Considerations regarding the feed-food competition between man and chicken

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List of abbreviations

ADF	acid detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
AIA	acid insoluble ash
BSE	bovine spongiform encephalopathy
BW	bodyweight
CA	crude ash
CF	crude fibre
CP	crude protein
DM	dry matter
EE	ether extract = crude fat
FAO	Food and agriculture organization (United Nations)
FCR	feed conversion ratio
GIT	gastro-intestinal tract
IMF	International Monetary Fund
ME	metabolizable energy
MJ	megajoules
N_{aa}	nitrogen calculated on amino acid content
N_{chitin}	nitrogen in chitin
N_{corr}	nitrogen corrected for nitrogen in chitin
NDF	neutral detergent fibre
NFE	nitrogen-free extract
$N_{Kjeldahl}$	nitrogen determined by Kjeldahl analysis
NMDS	nonmetric multidimensional scaling
N_{npnc}	non-protein non-chitin nitrogen
NRC	National Research Council
NSP	non-starch polysaccharides
OM	organic matter
OTU	operational taxonomic unit
SAR	South-African rand
SCFA	short chain fatty acids
S_{obs}	observed species richness
TMT	*Tarsometatarsus*

Definitions

Food	All human-edible ingredients, accepted to be eaten by people.
Feed	All ingredients that are fed to livestock, both human-edible and human-inedible.
Alternative ingredients	Human-inedible ingredients that can be used in feed.

Chapter 1

Introduction

Introduction

1.1. Feed-food competition

As the global population is growing, the need for food and feed is growing along. Therefore, an increasing competition for cereals and other human-edible ingredients between livestock feed and human food is taking place. Overall, the feeding of human edible sources to livestock is seen in intensive systems in developed countries, whereas the shortage of food is mainly located in developing countries (Erb et al., 2012). Increasing the use of human-inedible ingredients in the feed of livestock, on the one hand, and increasing the profit that rural livestock owners make on their animals, on the other hand, are two ways to temper this competition. Equally so has farming intensification allowed to supply food for over 7 billion people worldwide and, at the same time, the percentage of undernourished people has decreased from 18.7% in 1991 to 10.8% in 2015. The overall number of undernourished people in the world, however, is still close to 800 million (based on FAOSTAT, 2015) (**Figure 1.1.**). Therefore, finding the limit of decreasing the use of human-edible ingredients in the feed without tackling the production, will be important.

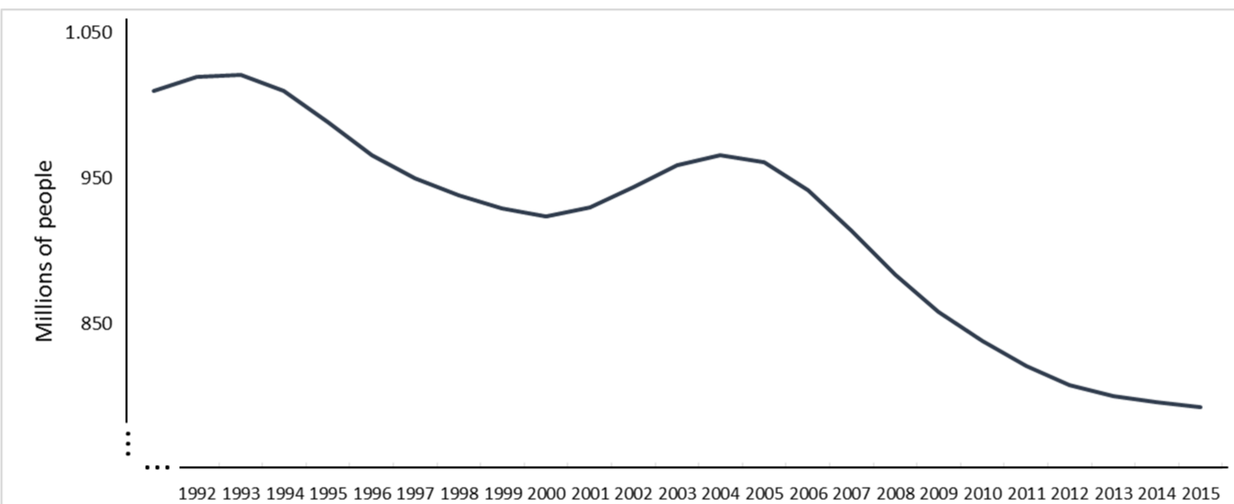

Figure 1.1. Global number of undernourished people over the years (FAOSTAT, 2015).

One third of the global production of cereal grains (1.3 billion tons a year) ends up in livestock feed and poultry production occupies about 8% of the world cereal grain production (Hinrichs and Steinfeld, 2007; Gustavsson et al., 2011). Taking into account that soybeans and cereal grains are human-edible and both cereals and soybean meal compose more than 80% of the chickens' diet, it is estimated that 75% of the broiler diet and 65% of the laying hens' diet is human-edible (Hendy et al., 1995; Gerber et al., 2007; Donohue and Cunningham, 2009; Wilkinson, 2011). The chickens' efficiency, however, to convert human-edible energy and protein from the feed to human-edible energy and protein in their meat and eggs, is often low. To estimate how much protein and energy was lost by consumption of animal protein, van Es (1975) was the first to calculate human-edible energy and protein conversion efficiencies.

$$\text{Energy efficiency} = \frac{\text{ME available for humans in animal products}}{\text{ME available for humans in animal feeds}}$$

$$\text{Protein efficiency} = \frac{\text{Protein available for humans in animal products}}{\text{Protein available for humans in animal feeds}}$$

This is a complex calculation and many different factors, such as the average age, the number of chickens dying during the production process, the amount of feed that is spilled and the feed consumed by the breeder animals, have to be taken into account. Therefore it is understandable that different results are obtained by different authors in different periods of time (**Table 1.1.**), the more as the feed conversion (kg of feed needed to gain one kg of bodyweight) of the chicken breeds improved over time (2.33 in 1975 *versus* 1.70 in 2010 (ACMF, 2013)) and it is up to the authors to decide whether a particular feed ingredient is regarded as human-edible or not. Furthermore, these efficiency calculations do not take the amino acid profile into consideration while plant foods are often limited in lysine (soybeans are an exception) and the most important amino acids (lysine, threonine, S-amino acids and tryptophan) are abundant in meat (Millward, 1999).

A human-edible protein efficiency higher than 100% was found for broilers in South-Korea. This means that during the whole production process, less kg of human-edible protein was available in the chickens' feed, compared to the eventual human-edible protein in eggs and meat. This is possible because these chickens are mainly fed human-inedible products. Boonen (2015) also obtained efficiencies higher than 100% as he considered soybean meal to be human-inedible because it is produced as by-product after oil extraction from soybeans. This is, however, debatable as soybeans get produced explicitly to be used in feed. One needs 1 kg of soybeans to produce 0.8 kg of soybean meal (Cromwell, 2012). As the diet of broilers contains 10% to >30% of soybean meal, the assumption that soybean meal has no food-value influences the efficiency results to a large extent. Overall are most efficiencies in **Table 1.1.** lower than one, indicating that human-edible protein and calories are lost by feeding concentrated diets to chickens.

Table 1.1. Human-edible protein and energy efficiencies of chickens converting feed to meat and eggs.

	Efficiency on human-edible energy	Efficiency on human-edible protein	Reference
Broiler	0.29	0.43	(van Es, 1975)
Laying hen	0.23	0.40	(van Es, 1975)
Broiler (Belgium)	0.19	1.00	(Boonen, 2015)
Laying hen (Belgium)	0.30	1.27	(Boonen, 2015)
Poultry (USA)	0.31	0.75	(Bywater and Baldwin, 1980)
Broiler (USA)	0.28	0.62	(Gill et al., 2010)
Broiler (South-Korea)	0.30	1.04	(Gill et al., 2010)

Still, food as feed for chickens might be beneficial in developed countries because production intensity is generally high and economic profits are highest for intensive chicken farms (Hinrichs and Steinfeld, 2007). Yet, when putting the feed-food debate in a global context, perspectives change. Different factors increase the demand for cereal grains globally (reviewed by Rosegrant and Cline, 2014) and meeting those demands will become challenging in the future as:

1) Human population growth is expected to increase the need for cereal grains for food.
2) Meat consumption per capita will increase, resulting in an increased demand for feed cereals.
3) The production of cereal grain for bio-fuel will increase, especially since fossil fuels are depleting (Leng, 2005; Donohue and Cunningham, 2009).
4) Climate change will decrease the suitability of agricultural land in some areas for crop production (Hutagalung, 2000; Pinotti and Dell’Orto, 2011; Rosegrant and Cline, 2014).

Higher grain prices do not only decrease accessibility to food for less affluent people, they increase the pressure on arable land and forest too (Foley et al., 2011). Currently, one third of all croplands are used to grow feed-crops (Steinfeld et al., 2006).

1.2. Possibilities for alternatives

Preliminary considerations

Pursuing maximal performances might not always be the best economical decision, nor the most ecological or socially accepted (Jackson et al., 1982; Wilkinson, 2011). Providing locally produced sources of feed, which are not useful in the human diet, can therefore be a more beneficial strategy for both domestic and industrial chicken farmers. Even when these feed alternatives contain a lower level of protein, a less protein-rich diet might not need to be compensated as protein guidelines for chickens are sometimes overestimated (Farrell et al., 1999a; Ravindran, 2012). And, when necessary, synthetic amino acids can be added to the diet (Becker and Wittmann, 2012). A reduction of more than 20% on the global warming potential of feed could be obtained by replacing soybean meal by peas and synthetic amino acids, even though the global warming potential of synthetic amino acids is higher than the one of soybean meal (Leinonen et al., 2013). In addition, broilers manage to regulate their protein intake to perform maximally when they are allowed to select their own diet from different ingredients. Not only by maximizing their protein intake, they can also limit their protein intake when the protein content in feedstuff is too high (Shariatmadari and Forbes, 1993; Gous and Swatsom, 2000; Kim, 2014). Furthermore, chickens are known to digest different nutrients better with increasing age (Batal and Parsons, 2002; Jiménez-Moreno et al., 2009), opening the opportunity to broaden the range of possible ingredients for feed at older ages. In addition, depending on the digestibility of the protein source, less protein in the chicken feed can reduce the nitrogen excretion and, therefore, be beneficial for the environment (Follett and Hatfield, 2001). For minerals and vitamins too, studies have shown that supplementation can be left out during the finisher period without affecting productivity or immunocompetence of chickens (Skinner et al., 1992; Khajali et al., 2006).

Further, the selection for high productive chickens happened in a certain setup with a concomitant evolution of feed, disease control and housing. Therefore, other characteristics such as the ability to deal with chitin or high fibre levels in the diet, feed selection or immunity against infections, might unconsciously have been lost in these chickens. When feeding alternative ingredients, however, different characteristics of the chickens might become important again. Slow growing and indigenous chicken breeds could thus be needed to increase the genetic diversity and to re-introduce certain features (Castellini et al., 2006; Grashorn, 2006).

Nevertheless, decreasing the use of human-edible ingredients in feed will not automatically make those ingredients available for human consumption. History shows that milk or grain surpluses in developed countries are rather destroyed to prevent a drop in prices, than being transported to developing countries or even distributed among the less fortunates in own country. Equally so, would dumping food surpluses in developing countries not be a sustainable solution either as it would tackle the local farmers and make those countries depend on import. In addition, the European legislation strictly distinguishes between feed-grade and food-grade ingredients to guarantee food safety. Unfertilized eggs from a chicken hatchery, for example, are not allowed in the food chain, even though eggs are consumable by people. These eggs can be used in feed but, again, only if certain criteria are fulfilled. When, for example, embryos are present in the eggs, they are not allowed in feed either (pers. comm. I. Kalmar). One way to contribute to the food availability in developing countries could be by using less imported ingredients. Soybean meal, for example, is mainly imported to Europe from South-America and Asia. Decreasing the use of soybean meal could therefore make local croplands available for food crops and attenuate deforestation. This argument too can be countered as it will decrease the income of soy farmers in those regions.

Alternative ingredients

Alternative ingredients in the chicken feed have extensively been reviewed (Ravindran and Blair, 1992; Farrell, 2005; Makkar et al., 2014) and numerous studies have proven the potential of many of those ingredients (**Appendix 1**). Cottonseed meal, for example, contains the metabolite gossypol, which inhibits digestive enzymes, reduces palatability and can, at high concentrations, be toxic to chickens (Morgan, 1989). Hence, cottonseed, from which the gossypol has been removed, can be fed up to 200 g per kg feed in broiler diets (replacing soybean meal and sorghum) without a loss of performance. For laying hens, a productivity of 90% was reached with the inclusion of 120 g and 200 g cottonseed meal per kg feed when the chickens were 18 to 57 weeks old (Perez-Maldonado, 2003).

Another example is meat and bone meal, which has already proven its value as a protein source in animal feed. Before bovine spongiform encephalopathy (BSE) caused an outbreak, meat and bone meal was the most important protein source in the poultry and livestock industry (Parsons et al., 1997). Although chickens can probably not transmit BSE from their feed to the consumer, the EU has banned all use of meat and bone meal in livestock feed to guarantee food safety (EC, 2001; Rodehutscord et al., 2002; Matthews and Cooke, 2003). Ever since, soybean meal has taken over as the most important protein source in feed worldwide, accounting for nearly 69% of all proteins used in animal feed (Cromwell, 2012). Strictly spoken, soybean meal can be considered a by-product, but when the calculations are made, it is clear that the use of soybean meal does not avoid feed-food competition. It takes 1 kg of human-edible soybeans to produce 0.8 kg of soybean meal. Using an average FCR of 1.6 and a meat: live bodyweight ratio of 70%, it is estimated that 2.3 kg of commercial feed is needed to produce 1 kg of broiler meat. Assuming that a third of the feed is soybean meal, 0.77 kg of soybean meal is needed for 1 kg of broiler meat. When converting this to human-edible soybeans, the soybean: broiler meat ratio is nearly 1:1. As soybeans are not the only human-edible ingredient in the broilers' diet and have a favorable amino acid composition, the use of soybean meal in feed does not increase the efficiency as calculated in **Table 1.1.** For laying hens, the soybean: egg ratio is much lower (0.24:1) but the diet of laying hens contains 50-60% of cereal grains.

A ton of meat and bone meal delivers the same amount of protein as 1.16 tons of soybean meal. Considering that it takes 0.44 million hectares to produce 1.16 million ton of soybean meal and meat and bone meal is a true by-product (Steinfeld et al., 2006; Wilkinson, 2011), it can be assumed that the reintroduction of meat and bone meal in the feed would, strictly spoken, increase the availability of soybeans in the human diet. Or, as soybeans are not highly demanded as food, the production of soybeans could be reduced and other crops or forest could replace soybean plantations.

Proposals of the EU commission to include animal derived protein back in the feed of monogastrics have been included in the roadmap 2010-2015. Since 2013, a PCR method to detect bovine DNA in samples was available and ever since animal-derived protein from non-ruminants are allowed in aquaculture. For poultry and pigs, PCR methods are being developed to identify the species of origin in the animal derived products. This is important in the context of article 11 of VO 1069/2009 to avoid cannibalism. Although these analyses are close to being available, two problems have been indicated. The first one is the nearly inevitable presence of cross contamination of the products and secondly, specifically for poultry, it appears difficult to find a communal DNA piece for all poultry species that can be used in PCR analyses (pers. comm. C. Keppens, FAVV).

Fibres

Defining nutritional fibre is not easy and many attempts have been done over time. Currently, dietary fibre is defined as "the remnants of the plant cells, polysaccharides, lignin and associated substances resistant to hydrolysis (digestion) by the alimentary enzymes" (DeVries, 2003). Divisions, within the group of dietary fibre, are made on nutritional and analytical characteristics, leading to entangled definitions of different fibre fractions (Hall, 2003) (**Figure 1.3.**). Plant cell walls are composed of non-starch polysaccharides (NSP) and lignin. Albersheim *et al.* (1984) classified NSP according to their solubility as cellulose, hemicellulose and pectin. When calculating or analyzing NSP, however, a part of resistant starch can be included too. Furthermore, based on the digestion and utilization, Van Soest *et al.* (1991) defined neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre (CF). The NDF includes hemicellulose, cellulose and lignin and ADF includes cellulose and lignin. Crude fibre measures a share of hemicellulose, cellulose and lignin (Van Soest et al., 1991). Depending on the sample, however, a part of the pectin, hemicellulose and cellulose might be in- or excluded from the NDF, ADF, CF or NFE fraction. Some pectins are even seen to appear in the ADF and not in the NDF (pers. comm. Sonja de Vries). Soluble and insoluble fibres are distinguished, based on their physiological effects (Prosky et al., 1988). Nitrogen-free extract (NFE), calculated as 100 – (crude ash + crude protein + crude fibre + crude fat + water), is not considered as a fibre fraction, but contains carbohydrates, sugars, starch and most of hemicellulose (Lloyd et al., 1978; Detmann and Valadares Filho, 2012).

Chitin is not a plant derived molecule, nevertheless, it can be considered as a polysaccharide as it is a polymer of N-acetylglucosamine, which is a glucose derivate (Richards, 1958). As chitin and the methods to analyse it are thoroughly discussed later, chitin is included in **Figure 1.3.**

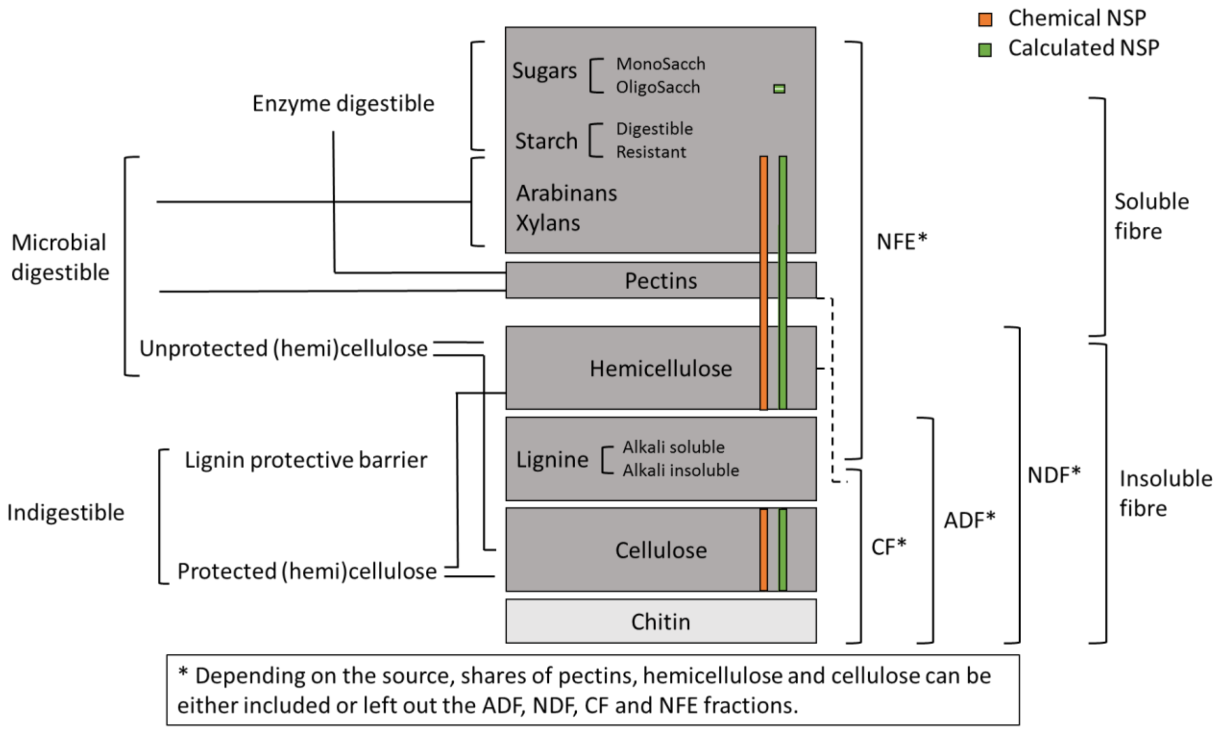

Figure 1.3. Overview of different fibre fractions (based on Lloyd et al., 1978; Prosky et al., 1988; Van Soest et al., 1991; Finke, 2007; McDonald et al., 2011; pers. comm. S. deVries).

Alternative ingredients are often high in fibre and therefore considered of low energetic value to the chickens' diet (Jørgensen et al., 1996). However, many beneficial consequences have been shown after inclusion of extra fibre in the diet.

- Whole triticale or wheat can be used in the diet to slow the growth rate of the chickens down by increasing the amount of fibre. This resulted in a reduced prevalence of ascites among the chickens (Jones and Taylor, 2001).
- Inclusion of inulin, high-fibre sunflower cake, oat hulls or soy hulls, improved the nutrient retention (Alzueta et al., 2010; González-Alvarado et al., 2007; Kalmendal et al., 2011).
- Improved feed conversion ratio was found after inclusion of both 3% oat hulls and 3% soy hulls (González-Alvarado et al., 2007).

- Oat hulls (mainly insoluble fibres) increased the passage rate in contrast to inulin (soluble fibre) which delayed it due to increased viscosity (Choct et al., 1999; Hetland and Svihus, 2001; Hetland et al., 2004; Alzueta et al., 2010).
- Inclusion of fibre in the diet increased the relative weight of the gizzard, the ceca and the overall digestive tract, whereas the length of the small intestine decreased (González-Alvarado et al., 2007).
- Equal feed consumption and egg production was shown with inclusion of 10% high-fibre sunflower meal in the laying hens' diet (raising the crude fibre level from 2.9% up to 5.3%) (Rezaei and Hafezian, 2007).

As demonstrated, different fibre fractions cause different effects on the health and production of the chickens, therefore, "fibre" should not be considered as a whole when studying its value in the chickens' diet. Yet, one must be aware that the results of the studies listed above are specific for the experimental setup mentioned. Other fibre inclusions, fibre sources or chicken breeds, for example, might give different results.

Microbiota

Although many fibre fractions are indigestible to the chickens' endogenous enzymes, cecal microbiota can break some of them down by fermentation (Dunkley et al., 2007). During fermentation, short chain fatty acids (**SCFA**) are produced which are proven to benefit the overall health and, more specific, the gut health of the chickens. Butyrate production, for example, is shown to reduce invasion and colonization of the gut by *Salmonella* and to stimulate intestinal epithelial growth (Pryde et al., 2002; Van Immerseel et al., 2005; Kien et al., 2007). Produced SCFA contribute to the nutrition of the chicken as they are absorbed across the mucosa of the ceca and catabolized, but this only contributes 3-4% to the overall energy that chickens extract from feed (Jørgensen et al., 1996). Moreover, SCFA lower the pH in the ceca and therefore protect the chicken against some pathogens and improve mineral absorption (Apajalahti, 2005; McWorter et al., 2009). Dunkley et al. (2007) incubated ten different high-fibre feed substrates with cecal content and measured the SCFA that were produced by fermentation of the substrates. Acetate production was highest, followed by propionate and butyrate. Bjerrum et al.

(2006) found the dominant bacteria in the ceca of both conventional and organic chickens to be related to *Faecalibacterium prausnitzii* and to produce butyric acid.

The chickens' gut microbiota is modulated by many factors, but one of the main factors remains the diet. A Finnish survey on the cecal microbiome of chickens on different farms reported that the profiles of the cecal microbiomes clearly clustered when farms were using the same diets, irrespective of the management used on the farms. The processing of the feed and the ingredients used are suggested to be equally important (Apajalahti et al., 2001). Especially fibre can influence the cecal microbiota, both in a beneficial as in a detrimental way. The risk for *Clostridium perfringens* colonization, which can cause necrotic enteritis (Van Immerseel et al., 2004), increases with the viscosity of the digesta. Therefore, diets rich in indigestible, water-soluble NSP, can trigger an *C. perfringens* infection (Kocher, 2003). Equally so have prebiotic effects been attributed to, for example, xylo-oligosaccharides in cereals or to lactose (reviewed by Hajati and Rezaei, 2010). Prebiotics have a positive effects on the gut health as they stimulate normal intestinal microflora and inhibit pathogens colonization. *Bifidobacterium*, for example, can get stimulated and therefore produces more acetate and lactic acid. Lactic acid decreases the pH in the intestine and prohibits, in that way, *E. coli* colonization (Patterson and Burkholder, 2003). The stimulating effect of prebiotics on the gut health of chickens is especially investigated since in-feed use of antimicrobials are prohibited by the EU in 2006.

Many studies have mapped the cecal microbiome of chickens (**Appendix 2**). Yet, it remains difficult to link overall microbiota shifts to changes in digestive capacity, production or gut health. Nevertheless, as the cecal microbiota is responsible for the fermentation of NSP, resistance against infection and improved gut health, their importance in chickens fed an alternative diet has to be acknowledged (Nurmi and Rantala, 1973; Dunkley et al., 2007; Eeckhaut et al., 2008).

So far, in all studies on the cecal microbiota of chickens, chickens were sacrificed in order to obtain samples. A method to obtain a cecal sample without the need of euthanizing chickens would make longitudinal studies easier and would reduce the sample size, therefore benefitting the welfare of the trial animals (Russell and Burch, 1959).

Insects

Insects are natural converters of organic waste streams into protein and fat, and they are part of the natural diet of chickens (Goromela et al., 2006). Including them as an alternative ingredient in the chickens' diet would decrease the feed-food competition for different reasons:

1. When compared to grain, they need less space per kg protein. Resulting in a higher protein yield per km² (Oonincx and de Boer, 2012).
2. Some insects have a reproduction cycle of a few generations per year.
3. A high variability of edible insects has been reported: more than 1500 species of edible insects in 113 countries (Jongema, 2015).
4. Overall, insects have a good nutritional value for protein, fat, vitamins and minerals (especially Fe and Zn) (Bukkens, 1997; Finke, 2007; Ramos-Elorduy et al., 2011; Oonincx and Dierenfeld, 2012).

Including insects in the feed, however, also arises some challenges. As huge insect quantities will be necessary, as pesticides might be present on insects caught from the wild (Banjo et al., 2006) and as overharvesting will affect the insect population and the whole environment (Yen, 2010; Ramos-Elorduy et al., 2011), it will be important to breed insects in controlled environments. Hence, research into breeding protocols and management policies will be necessary. In addition, breeding insects might raise questions on insect welfare (Eisemann et al., 1984). Furthermore have allergic reactions to insects been described, mainly caused by chitin (Linares et al., 2008; Verhoeckx et al., 2014) and some insects are reported to be toxic (Blum, 1994; Adamolekun et al., 1997; Zagrobelny et al., 2009). Other risks such as disease transmission and bad preservation of the feed (Blum, 1994; Schabel, 2008; Zagrobelny et al., 2009; Klunder et al., 2012) also need to be studied.

The European legislation on the use of insects in feed and food is unclear. As insects are not explicitly mentioned as feed or food ingredient in the law, one can speculate which rules apply to them. The two main questions that need to be answered are: "do insects belong to the group of novel foods"? In other words: "have they been eaten in Europe to a "significant degree" before May 15, 1997"? And secondly: "should insects and their products be considered animals and animal-derived products"? The EU legislation about the use of animal derived products in the feed of production animals is complex. Whether insects are considered animals or not will affect the criteria that need to be met before being allowed in feed. Including insects in the group of "novel foods" will complicate and postpone the approval to use insect products in food (van der Spiegel et al., 2013; Eeckhout and Verbeke, 2015). Awaiting a clear opinion in the EU, the FAVV has allowed ten insects for human consumption within Belgium. These ten can be sold as long as the principles of food security are respected and the company is registered with the FAVV (FAVV, 2014).

As food, insects are consumed in many countries. Therefore, one can wonder whether insects will not contribute to the feed-food competition. As people in Europe and the USA are overall reluctant towards the idea of consuming insects (Mitsuhashi, 2010; Megido et al., 2014), it can be expected that the competition will rather be small in these regions. If insects for feed could be bred on absolute waste streams such as kitchen leftovers, garden waste or manure, they can be considered as by-products of waste processing. Moreover, the use of insects as food is ecological more beneficial than the consumption of meat. When compared to livestock, insects excrete less greenhouse gasses, have a more optimal feed conversion and their production process needs less water (**Figure 1.4.-1.5.**). Breeding more insects because of a competition between feed and food for insects would therefore be more sustainable compared to an increased consumption of beef, pork and chicken.

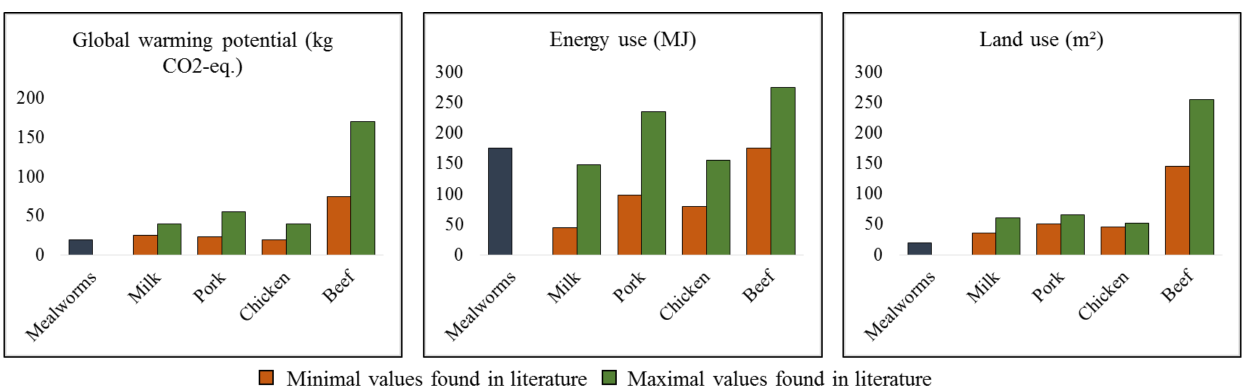

Figure 1.4. Greenhouse gas production (global warming potential), energy use and land use due to the production of 1 kg of protein from mealworms, milk, pork, chicken and beef (adapted from Oonincx and de Boer, 2012)

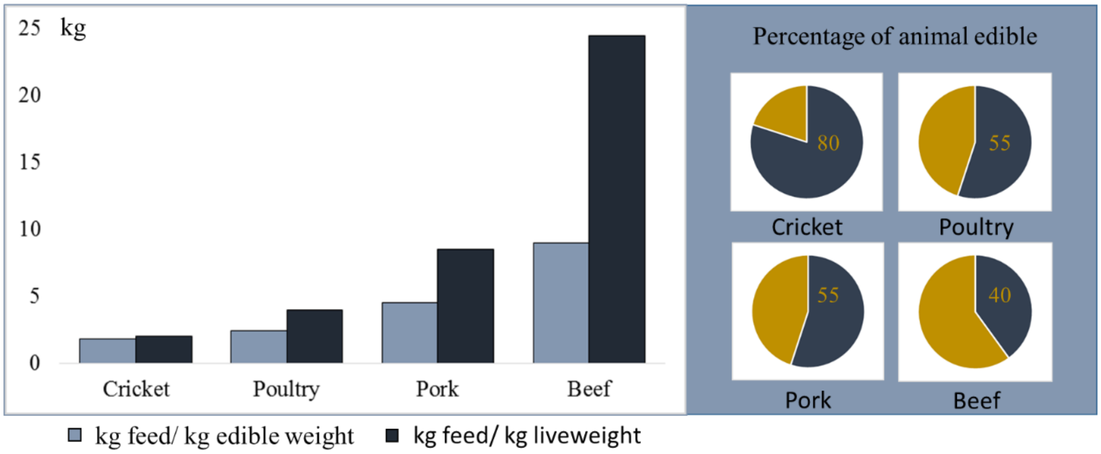

Figure 1.5. Efficiencies of production of conventional meat and crickets (adapted from van Huis, 2013)

To study the nutritional composition of insects or the digestibility of feeds containing insects, specific methods will be needed as chitin is present in insects. Chitin is a polysaccharide included in the procuticle of insects where it is an important part of the exoskeleton (Richards, 1958). Specific methods are needed as chitin and protein both contain nitrogen and the crude protein analysis is based on the determination of total nitrogen by the Kjeldahl method. Equally so are the methods to determine chitin (Finke, 2007; Liu et al., 2012) close to the methods to analyse fibre fractions (Prosky et al., 1985; DeVries, 2003). In diets composed of both animal and plant derived ingredients, distinguishing between chitin and crude fibre on the one hand and between chitin and protein on the other hand, proves challenging (Finke, 2007).

1.3. Evolution of the chicken

The origin of chickens

The taxonomy ("science of classifying living things") and the reconstruction of the origin of domestic chickens has not been easy (Liu et al., 2006). Today it is accepted that they belong to the order of the *Galliformes*, family *Phasianidae* and genus *Gallus* which equals the genus of the junglefowl (Crawford, 1990a). Four different species of junglefowl can be distinguished: red junglefowl (*Gallus gallus*; previously known as *G. bankiva* and *G. ferrugineus*), grey junglefowl, green junglefowl and Ceylon junglefowl. Based on geographical location, different sub-species exist within the species of red junglefowl. This species is considered the main ancestor of the domestic chicken (Moreng and Avens, 1985; Crawford, 1990a; Fumihito et al., 1994; Moiseyeva et al., 2003), although some authors suggest that the grey junglefowl (*G. sonneratii*) also contributed (Tixier-Boichard et al., 2011).

Today, red junglefowl still occur in the wild in South-Eastern Asia (India, China, Java, Malaysia, Indonesia, and the Philippines). They are mostly found in undisturbed mixed forests in the tropics and sub-tropics but can also be found on field edges, groves, plantations and scrubland (Ali and Ripley, 1989; Delhoyo et al., 2001). The habitat flexibility minimizes the direct negative effect of forest loss on its conservation. Other threats to the red junglefowl include predation, poaching and egg collection, but the biggest reason for the decreasing number of pure red junglefowl is the genetic loss through crossbreeding with domestic and feral chickens (Ali and Ripley, 1989; Peterson and Brisbin, 1998). The original purpose of domesticating chickens was game and entertainment rather than meat and eggs. West and Zhou (1989) suggest that chickens were domesticated in Southeast Asia way before 6000 BC and later they were introduced in Northern China where chicken bones, larger than the ones of the wild red junglefowl, were found. Proof of domestication of chickens in India only dates from 2000 BC and it is not clear whether this has been introduced from Southeast Asia too, or was an isolated event. Illustrations of fighting roosters and little statuettes out of clay were found in the Indus Valley (2500 BC). With the Romans, keeping poultry was well established and they started using them as a food source, though they were still used in religion and entertainment (West and Zhou, 1989; Crawford, 1990a).

Thousands of years have passed between the domestication of the chicken and the situation of today where industrial broilers achieve 2.77 kg of bodyweight in 42 days (Aviagen, 2012) and laying hens produce 413 eggs in 72 weeks (Genetics, 2014). However, the development of the poultry industry is a recent phenomenon, happening in approximately hundred years' time. The current industrial breeds originate from only a few "old" breeds with extraordinary traits in meat or egg production. Though many different chicken breeds were used all over the world, domestication and industrialization caused a decreasing genetic diversity and care must be taken in order not to lose interesting traits and features seen in less productive breeds (Al-Nasser et al., 2007). Indigenous chickens, mainly kept in developing countries, Mediterranean egg-type and the true Bantam, have been proven very important breeds in this matter (Moiseyeva et al., 2003).

Unconscious selection

The first chapter of Darwin's "*Origin of species*" addresses "*The variation of animals and plants under domestication*". In this chapter Darwin describes the effect of human selection on different organisms, including chicken (Darwin, 1859). Ever since chickens have been domesticated, broilers and laying hens have been selected for different traits. Domestication changed some aspects of phenotype, behavior and physiology extensively (Zulkifli et al., 1999; Schütz et al., 2001; Kerje et al., 2003), but Darwin distinguished between conscious and unconscious selection (Darwin, 1859). Broilers, for example, have been selected for rapid growth, low feed conversions and heavy breast muscles. As these are the desired traits in order to increase the productivity and therefore the economic benefits, these traits are part of the conscious selection. However, when these highly selected chickens are compared to their initial ancestors, the red junglefowl, it appears that they also have smaller brains, lighter bones and smaller lungs (Vidyadaran et al., 1990; Jackson and Diamond, 1996). These are assumed to be the result of unconscious selection.

Comparing different traits between commercial chickens and the red junglefowl raises the question whether, over the years, chickens have not been (unconsciously) selected to high performance on energy-rich diets, rather than on high performance no matter what the feed is. They might show a superior feed conversion when fed the commercial diets, but might lose their lead on low energy and high fibre diets.

1.4. Industrial chickens

Broilers and laying hens

Although different breeds can be kept for both meat (broilers) and egg production (laying hens), most of the industrial chickens belong to a few breeds only. The heavy, fast-growing broilers originate mainly from Cornish breed and White Plymouth Rock. Three current breeds represent the biggest part of broilers in the world: Cobb, Ross and Hubbard (Aviagen; Cobb-Vantress; Groupe Grimaud). Concerning the laying hens, white eggs mainly come from single comb White Leghorns and brown eggs from Plymouth Rocks, New Hampshire, Rhode Island Red and Australorp (Crawford, 1990b; Siegel et al., 1992), illustrating the narrow genetic origin of both types of commercially used chickens.

Diet

It is not easy to determine specific guidelines regarding the required nutrients in a chicken diet because:

1. The required levels of the nutrients vary between laying hens and broilers, between management policies and evolve during the production process.
2. The optimal composition of a diet is affected by the specific situation. Factors such as climate, stocking density and chickens' genetics interact.
3. The quality of the ingredients can differ between different batches.
4. The legislation can forbid the use of certain ingredients (cfr. meat and bone meal).
5. Only minimum requirements for crude protein, amino acids, minerals, vitamins, energy, and linolenic acid are described in the "nutrient requirements for poultry" (NRC, 1994).
6. There is a lack of specific guidelines for different fibre fractions, carbohydrates and fatty acids other than linolenic acid, even though the beneficial effects of calculated supplementation of some of those fragments have clearly been proven (Alzueta et al., 2010; Kalmendal et al., 2011).
7. Commercial diets are composed on cost-benefit analyses and therefore do not necessarily represent the optimal diet regarding chicken health, welfare or even growth.
8. Feed companies keep the results of their trials secret.

Moreover is the NRC, considered the reference for poultry nutrition, fairly outdated (NRC, 1994). Recent studies have questioned the guidelines in the NRC and have shown better productions by different amino acid or micronutrient inclusions. Sakomure et al. (2015), for example, re-determined amino acid guidelines for broiler breeders and found levels that differed from the NRC guidelines. Leeson (2007) re-evaluated vitamin requirements and found, for example, that vitamin E levels should exceed NRC requirements for optimal production and health.

1.5. Rural chickens

Chickens are the number one livestock kept in rural areas (Alders and Pym, 2009), but only few statistics are available on the productivity and management of chickens in developing countries. Still, based on the published numbers, some estimates can be made. Indigenous breeds probably represent 70-95% - with an average of 80% - of the national chicken flock in developing countries (Guèye, 1998; Goodger et al., 2002). Because of their low productivity, however, the contribution they make to the national production of eggs and meat is expected to be much lower. Nevertheless, the FAO estimates the rural human population to count 3.36 billion heads (or 46% of the global population), indicating that rural chickens should be considered very important (FAOSTAT, 2015).

Production

The number of eggs delivered for consumption by an indigenous hen per year lies between six and sixteen. Hence, not all of the laid eggs are kept for consumption or sale. This estimation was made by Pym *et al.* (2006) based on the production numbers of two to four clutches a year, each containing about ten to sixteen eggs (Sonaiya et al., 1999; Henning et al., 2007). Over 80% of the eggs are bred by the hen, delaying the next clutch of eggs and decreasing the number of eggs available for consumption (Sonaiya et al., 1999; Khalafalla et al., 2002; Njue et al., 2002; Pym et al., 2006; Henning et al., 2007). Hens hatching their eggs should not necessarily be a disadvantage if it was not that only a low number of chicks survive (Aini, 1990; Cumming, 1992; Sonaiya et al., 1999). Other causes for the low production number of eggs can be looked for in the genetics of the indigenous birds, seasonal effects and the low concentration of some nutrients and minerals in their diet (Tadele, 1996; Goromela et al., 2006). When comparing the indigenous breeds with hybrid layer hens (6 to 16 versus 250 to 300 eggs per year, respectively), Pym *et al.* (2006) estimate that the contribution of indigenous chickens to total egg consumption in developing countries is only 8%. For meat, the contribution of indigenous chickens is estimated to be higher; 67% of the chickens slaughtered for meat in developing countries comes from indigenous breeds. When correcting for the difference in bodyweight (0.8 versus 1.5 kg), this number decreases to 50% (Pym et al., 2006).

Management

When leaving the industrial systems aside, poultry management in developing countries can be divided in four systems: the free-range system, the backyard or semi-scavenging system, the semi-intensive system and the small-scale intensive system (**Table 1.2.**). The first one is used by most of the rural families. One to ten indigenous chickens, with a growth rate between 5 and 10 g/ day, are kept around the house without specific shelter. The input in such a system is low since little to no labor or money is put into it. In other systems, feed is often the most expensive input (up to 70% of the production costs (Hinrichs and Steinfeld, 2007; Donohue and Cunningham, 2009; Yegani and Korver, 2013)) but chickens in a free-range system roam around freely and forage for their own feed. In that way, even poor families who cannot afford to buy or share their food with the chickens, can benefit from keeping a few chickens. A downside consequence of this "low-input system" is the high mortality and the low productivity due to different factors such as predation, infectious diseases and parasites (Kusina et al., 1999; Alexander, 2001; Swai et al., 2013).

Table 1.2. Different chicken management systems in developing countries, with the traditional free-range management being the most frequently used.

	Traditional free-range	Backyard or subsistence	Semi-intensive	Small-scale intensive
Flock size	1-10 birds	10-50 birds	50-200 birds	50-500 birds
Key rearers	Majority of rural families	Moderate number of rural families	Few rural families	Urban families
Ownership	Mostly women & children	Mostly women & family	Middlemen	Business men
Type of breeds	Indigenous breeds	Indigenous & few crossbreds	Local/ improved	Layers or broilers
Feed resources	Scavenging	Scavenging & supplementation	Commercial/ local	Balanced diets
Health status	No vaccination/ medication	Vaccination & little medication	Vaccination	Full vaccination
Housing system	No specific housing	Simple & small houses	Medium & improved	Big & improved
Egg production	30-50/(year × hen)	50-150/(year × hen)	80-160/(year × hen)	250-300/(year × hen)
Growth rate	5-10g/ day	10-20g/ day	10-20g/ day	50-55g/ day
Mortality rate	High mortality	Moderate mortality	Low mortality	Low mortality
Use of products	Home consumption	Home consumption & sale	Family income	Business income
Profit	Small cash income	Family income	Family income	Business income
Socio-economic	Social and cultural	Social & micro-credit	Credit based assets	Little social

Based on (Sonaiya et al., 1999; Guèye, 2003; Riise, 2004; Goromela et al., 2006; Sonaiya, 2007).

Scavenger diet

Rural chickens, kept in a completely extensive way, have to find their feed by foraging in the environment (**Figure 1.6.**). Many different ingredients were found in the diet of scavenging chickens (Goromela et al., 2006), both of human and natural origin. Goromela *et al.* (2006) found an average nutrient composition of 100 g crude protein/ kg DM, 11.2 MJ/ kg DM, 11.7 g Ca/ kg DM and 5 g P/ kg DM in the crop content of scavenging chickens. They concluded that these nutrient concentrations were too low to support high productivity. However, this conclusion was based on the comparison with concentrations needed for broilers or layers in the industry (NRC, 1994). No information was given about the absolute amount of feed per day that was ingested by the birds.

Figure 1.6. Indigenous chickens roaming freely (Limpopo, South-Africa and Kabale, Uganda).

Chapter 2

Aims and objectives

Aims and objectives

As the global population is growing, the need for food and feed is growing along. Therefore, an increasing competition for cereals and other human-edible ingredients between livestock feed and human food is taking place. Overall, the feeding of human edible sources to livestock is seen in developed countries whereas the shortage of food is located in developing countries. Increasing the use of human-inedible ingredients in the feed of livestock, on the one hand, and increasing the profit that rural livestock owners make on their animals, on the other hand, are two ways to temper this competition.

The general aim of this Ph.D. dissertation is to regard the problem from a helicopter view in order to get a global idea about the problems that play. In order to contribute to solutions in the future, points of focus and critical factors for further research were identified.

1) The first objective of this PhD dissertation is to get an idea about alternative ingredients and diet composition by observing the diet of rural, free-range chickens.

From this first observation, two considerations were selected. The first consideration assumes that many alternative ingredients will contain a high fibre content. The second one brings up insects, natural converters of waste into protein and fat, as a promising alternative ingredient in the future. Hence, to study the chickens' ability to break fibres down, the cecal microbiome of chickens will be crucial. Furthermore, the digestibility and nutrient composition of samples that contain insects will need to be studied. Therefore, two additional objectives of this dissertation are:

2) To develop a method for longitudinal cecal micriobiome studies in chickens.

3) To develop a more accurate method to analyse the protein content in samples that contain insects and therefore chitin.

To make alternative ingredients applicable in the industry, information about the productivity of chickens is crucial. Therefore, the last aim is:

4) To investigate the flexibility of chickens to perform on a less concentrated diet

Chapter 3

Review on the diet of free-ranging chickens in developing countries

Review on the diet of free-ranging chickens in developing countries

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Abstract

Rural chicken owners can often not afford to feed grains to their chickens. Industrialized chicken production, moreover, is highly competitive for grain feed that might be used directly for human food. To increase the production of rural chickens in a sustainable way and to decrease the feed-food competition, identifying alternative feed resources offers opportunities. Observing the free-choice diet of free ranging chickens draws attention to new ingredients and explains how these ingredients steer the nutrient profile. A literature survey was made on free ranging chickens and the overall nutrient composition of the crop content was compared with an average commercial diet. A wide variety of ingredients was found in the crops. The average commercial diet was lower in crude fibre, higher in crude protein, Ca, P and energy and tended to be higher in ether extract compared to the free-range diets. In the crops of free ranging chickens, cereals and seeds were the most abundant ingredients. The amount of seeds was positively correlated with the crude fibre content.

Introduction

The competition for cereals between feed, food and fuel is a current issue (Donohue and Cunningham, 2009; Foley et al., 2011). While most of the produced cereals are acceptable for human consumption, one to two thirds (globally and in developed countries respectively) end up in livestock feed (Erb et al., 2012). About 75% of the broilers' diet and 65% of the laying hens' diet is estimated to be consumable by mankind as the global average diet of a chicken is estimated to contain more than 60% of cereals (Hendy et al., 1995; Gerber et al., 2007; Wilkinson, 2011).

For broilers in the USA, it is estimated that converting chicken feed to chicken meat reduces the human-edible energy and protein with 72% and 38% respectively. In South-Korea, however, the human-edible protein and energy content in poultry meat is higher than the feed consumed by the chickens due to the use of human-inedible feed (Gill et al., 2010; Teja Tscharntke et al., 2012). This raises the question why feed consumption of domesticated animals has shifted to food-grade ingredients. Especially since many of them descend from animals feeding on a wide variety of ingredients (Arshad et al., 2000).

In low income countries, 70 to 95% of all chickens are slow growing (Huque, 1999; Pym, 2008) and self-reliant to find feed (Savory et al., 1978). Knowledge about the feed resources of free ranging chickens and the concomitant dietary nutrient profile will identify potential feed ingredients that do not compete with the human diet and are available in the environment of the chickens. In addition, information about how the ingredients in a free-range diet steer the nutrient profile will be achieved and this nutrient profile will be compared to a standard commercial diet.

Material and methods

Google Scholar was searched for articles evaluating the ingredients and nutritional composition in the crops of scavenging poultry. Now the keywords included *"crop content, gastrointestinal content, scavenging, scavengeable feed, feeding behavio(u)r, feeding habits, feed resources, free-range or nutritional status"* AND *"chickens, poultry, broiler, hen, guinea fowl, junglefowl or jungle fowl"*. Only articles where the entire crop content was analyzed for ingredients and/or nutritional composition and with free feed-choice for the chickens were taken into account. It was decided to pool the results of different genotypes and different regions. Datasets in the same study but obtained from different seasons were considered separate datasets.

Secondly, a database was created based on twenty basal diets described in peer reviewed articles found on Google Scholar using the keywords *"commercial, control, base, industrial and standard"* in combination with *"diet, feeding or feed AND poultry, broiler(s), laying hens or chicken"*. Broilers and laying hens were equally represented in the studies. For laying hens, the diets for chickens older than eighteen weeks were used, based on the ages of the free ranging birds in the reviewed articles. Since the maturation rate of commercial broilers differs from the rural dual purpose breeds, the industrial diets for broilers in the grower phase were considered, although the free ranging broilers are estimated to be older than 35 days.

When crude protein (CP), ether extract (EE), crude fibre (CF) and crude ash (CA) were given, nitrogen-free extract (NFE) was calculated as: $NFE = 100 - (CP + EE + CF + CA)$ (with CP, EE, CF and CA presented as a percentage of dry matter (DM)).

In order to calculate the correlations with the nutrients, the ingredients found in the crops of the free-ranging chickens were separated in two groups: supplemented and environmental feed. Supplemented feed covers: oil seeds and cakes, brew wastes, kitchen wastes, cereal bran and cereals. Environmental feeds covers: tree, grass and fruit seeds, green forages, invertebrates, and sand and grit. Correlations between the nutrients and the ingredients in the crop contents were calculated using Spearman correlation. Nonparametric one-way Kruskal-Wallis rank test and the Dunn post hoc test were used to find the differences in nutrient composition between the commercial broiler and laying hen diets and the crop contents of the free ranging chickens (SPSS 22.0, IBM SPSS Statistics Inc., USA).

Results

Twelve studies (representing twenty-six datasets) on the crop content of free ranging chickens were withheld from the selection (**Table 3.1.**). Fourteen other studies matching the keywords were found but did not match our selection criteria (see **Appendix 3**).

The nutritional comparison of the commercial diets (references listed in **Appendix 4**) with the crop contents of free ranging chickens revealed no differences between the three diets for ether extract, crude ash, phosphorus and NFE concentrations ($P > 0.05$). The ME level was higher in the broiler diet compared to the free-range diet ($P = 0.010$). The Ca concentration was higher in the diet of the laying hens compared to the free-range diet ($P = 0.002$). Crude fibre was highest in the free-range diet compared to the broiler diet ($P = 0.012$) and tended to be higher than the diet of the laying hens ($P = 0.052$). The free-range diet had an overall lower CP concentration ($P \leq 0.005$) compared to both commercial diets (**Figure 3.1.**).

When the nutrient compositions of both commercial diets were put together as one average commercial diet and compared to the free-range diets, the commercial diet was higher in crude protein (18.6 ± 2.1 *versus* 11.0 ± 2.7%; $P < 0.001$), Ca (2.3 ± 1.4 *versus* 1.1 ± 1.0 %; $P = 0.002$) and energy (12.6 ± 1.1 *versus* 11.6 ± 1.6 MJ/ kg; $P = 0.031$), it tended to be higher in ether extract (4.5 ± 2.3 versus 6.2 ± 2.4%; $P = 0.050$) and P (0.5 ± 0.2 *versus* 0.4 ± 0.2%; $P = 0.050$) and was lower in crude fibre (4.1 ± 1.6 versus 7.5 ± 2.4%; $P < 0.001$).

Table 3.1. Articles used in the literature review.

Article	Country	Sample size	Age and type of chickens	Period
(Dahouda et al., 2008)	Benin	120 guinea fowls	8-12 months	dry and rainy season
(Dessie and Ogle, 2000)	Ethiopia	270 chickens	laying birds, 7-8 months	dry, short rainy and main rainy
(Goromela et al., 2008)	Tanzania	648 chickens	not indicated	dry and rainy season
(Gunaratne et al., 1993)	Sri Lanka	375 chickens	Chicks, pullets, cockerels and laying hens	Not indicated
(Huque, 1999)	Bangladesh	500 chickens	laying hens	dry season (= winter & summer) and rainy
(Mekonnen et al., 2009)	Ethiopia	208 chickens	104 layers, >1 cycle 104growers, 3-5 months	harvesting and non-harvesting period
(Minh et al., 2006)	Vietnam	384 chickens	dual purpose 20 weeks (point-of-lay)	dry and rainy season
(Momoh et al., 2010)	Nigeria	158 chickens	54 layers, >26 weeks 104growers, 3-5 months	early rainy, late rainy, early dry and late dry
(N. a Mwalusanya et al., 2002)	Tanzania	144 chickens	72 hens, >1 laying cycle 72 growers, 2-4 months	short rainy and long rainy
(Peters et al., 2010)	Nigeria	120 chickens	3 genotypes dual purpose11 >1 breeding cycle	dry season
(Pousga et al., 2005)	Burkina Faso	128 chickens	dual purpose, 5-6 months	dry and rainy season
(Rashid et al., 2005)	Bangladesh	100 chickens	50 layers, >26weeks 50 growers, 3-5 months	harvesting period

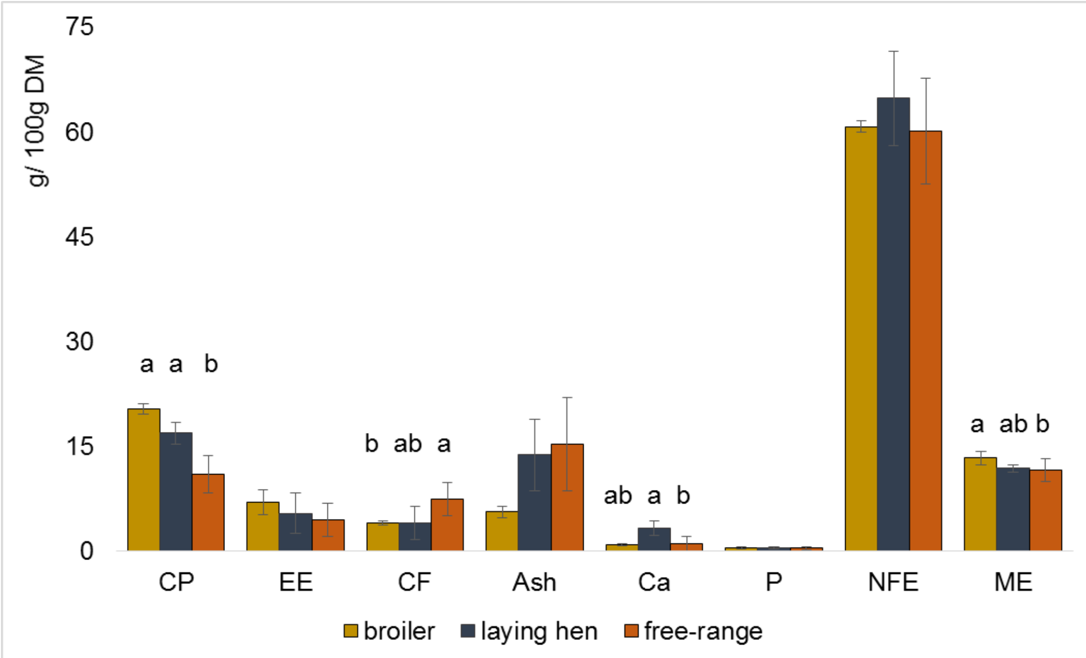

Figure 3.1. Nutrient composition of three diets: commercial broiler, commercial laying hen and free-range diet. Crude protein (CP), ether extract (EE), crude fibre (CF), Ash, Ca, P and NFE (nitrogen-free extract) are presented as percentages (10g/ kg). Error bars represent standard deviations. [a,b] Different letters indicate significant differences between groups at $P < 0.05$. Based on Table 3.1. and Appendix 4.

Based on the literature review on the crop content of scavenging chickens these ingredients were found (n is the number of studies out of 26 that reported the ingredient). Grains (median: 34%; n=17) and tree, grass and fruit seeds (median: 28%; n=8) were predominantly found in the crops of free ranging chickens, followed by kitchen waste (median: 18%; n=19), cereal bran (median: 14%; n=6) and green forages (median: 10%; n=22). Oil seeds and cakes (7%; n=2), invertebrates (insects, millipedes and worms) (median: 6%; n=20), sand and grit (median: 5%, n=6), eggshells (median: 4%; n=2), bones (median: 4%; n=2), beans (median: 1%; n=3), nuts (median: 1%; n=4) and brew wastes (median: 3%; n=4) were also reported.

In the literature database, season did not affect the ingredients or any of the nutrients found in the crop content of free ranging chickens (all of them $P > 0.05$). Therefore all results were reorganized together in one large dataset. Correlations between ingredients and nutrients were calculated (**Table 3.2.**). A negative correlation was found between the proportion of environmental feed and the concentration of dry matter (r = - 0.410; $P = 0.038$; n = 26) in the crops of free ranging chickens.

Table 3.2. Correlations between ingredients and nutrients in the crop content of free ranging chickens.

	Ingredient	Nutrient	*P*-value of correlation	r	N
Supplemented ingredients	Kitchen waste ↑	Ca ↑	0.010	0.575	19
Environmental ingredients	Seeds ↑	CF ↑	0.004	0.881	8
	Green forages ↑	DM ↓	0.007	-0.558	22
		Ca ↑	0.026	0.472	22
	Insects, millipedes and worms ↑	DM ↓	0.009	-0.567	20
		Ca ↑	0.044	0.454	20
		Ash ↓	0.047	-0.475	18

The nutrients are represented as dry matter (DM), crude fibre (CF), crude ash (Ash) and Ca. Based on (Gunaratne et al., 1993; Huque, 1999; Dessie and Ogle, 2000; N. a Mwalusanya et al., 2002; Pousga et al., 2005; Rashid et al., 2005; Minh et al., 2006; Dahouda et al., 2008; Goromela et al., 2008; Mekonnen et al., 2009; Momoh et al., 2010; Peters et al., 2010).

Discussion

Large differences appeared among the crop contents in the different studies. In general, kitchen or household wastes and grains represented a big part of the reviewed crop contents, suggesting that the larger share of the free-range diet was provided by humans. Nonetheless, conclusions must be made carefully as chickens are known, for example, to pick the remaining grains from cattle litter and manure (Faouzi et al., 2000). The same applies to animal bones, found in the crop content of non-supplemented red jungle fowls (Arshad et al., 2000). Chickens have been observed to consume birds caught by cats and even to catch and kill birds themselves (McBride et al., 1969). Another abundant ingredient was seeds. The amount of seeds was positively correlated with the concentration of CF but this did not stop the chickens from ingesting them, though, chickens are known to extract only a negligible amount of energy from NSP (Jørgensen et al., 1996). This is in line with the study where the growth of slow growing chickens was not hampered by high dietary fibre levels (Pauwels et al., 2015). Insects, millipedes and worms represented only a smaller share in the crop contents of free ranging chickens, yet four out of five studies reported their presence. Arshad *et al.* (2000) indicated that most of the invertebrates found in the red jungle fowls' crops could not fly and therefore could easily be caught by the fowls. Olukosi and Sonaiya (Olukosi and Sonaiya, 2003) only used the number of crawling insects to predict the quantity of scavengeable feed for poultry. Nevertheless, McBride *et al.* (1969) observed chickens to chase and catch flying insects.

Cereals often make up the bulk of the crop content. When comparing the free-range with the commercial diets, human interference induced a shift from a fibrous, low-protein and low-energy diet to a low-fibre, high-protein and energy-rich diet. One could assume that this shift is mainly due to the higher energy and nutrient demands of the high-performance commercial breeds, but the phenomenon is already seen when people start supplementing rural scavenging chickens. This raises a "chicken or egg" question: is the actual nutrient and energy profile of feeds for commercial breeds a consequence of the increased requirements for fast growth or high egg production, or did these breeds develop based on the diet change that was induced by humans?

No correlation between any of the ingredients and NFE could be found. Although it would have been interesting to evaluate the differences in starch and sugar versus more fibrous components within NFE between the commercial and the free-ranging diets. These analyses were not reported in the studies reviewed. The analysis of NFE should not be considered sufficient to estimate the nutritional value of this fraction for chickens, because the sub fractions contained in NFE can range from enzymatically digestible nutrients such as sugars and starch up to more fibrous components such as pectin in plants (Saura-Calixto et al., 1983; Malathi and Devegowda, 2001; Weurding et al., 2001).

Since CF is accepted to be of low energy value to chickens (Jørgensen et al., 1996), it is kept low in the commercial diets and enzymes like xylanase are often added to break non-starch polysaccharides down (Choct, 2006). Both positive and negative effects of CF on the digestibility of other nutrients have been shown (Jørgensen et al., 1996; Hetland et al., 2004). Apart from digestibility, CF in the diet also decreases cannibalism, proventricular dilatation and mortality due to ascites (Jones and Taylor, 2001; Hetland et al., 2004). The idea of keeping chickens away from fibre-rich diets might thus have to be revised. It appears that scavenging chickens do not avoid fibrous components, hence they might have developed a way to deal with the intake of high-fibre low-energy diets. A higher fibre digestibility has been found in slow growing chickens (Label Rouge) compared to fast growing chickens (Cobb) (Kras et al., 2013).

Because of the lack of energy and nutrients in soil and sand, it might be concluded that this should be avoided when feeding commercial breeds. However, an average of 5.8% sand and grit has been found in the crop of free ranging chickens, indicating the importance of this ingredient in the diet of chickens. Gull *et al.* (2014) linked geophagia – the intended ingestion of soil and sand – to the provision of minerals, detoxification and other applications. Only six out of twenty-six datasets reported the presence of sand and grit, but this ingredient is expected to be often included in the fractions “others” or “undefined”.

Rather than absolute numbers, concentrations of nutrients and ingredients in the crop content of free ranging chickens were reported which might explain that no effect of season on the crop composition was found. In contrast, farmers in Ethiopia noted that feed was more available in the dry season (Desta and Wakeyo, 2012) and a study in Burkina Faso stated that supplementing the diet at the end of the rainy season was not necessary (Kondombo et al., 2003). Several other factors such as age (Savory et al., 1978), sex (Arshad et al., 2000), genotype (Minh et al., 2006) and supplemented diet (Horsted et al., 2007) have been mentioned to affect the crop composition of free ranging chickens.

The difference in energy content of the commercial diets and the free-range diet must be interpreted carefully. Many studies on the crop content of free ranging chickens calculated ME by using the Wiseman formula (Wiseman, 1987) though this formula uses a constant factor of 3951, based on an average for commercial diets containing high starch contents. This might induce an overestimation of ME in free-range diets. Moreover, although the ME level per kg can be similar between both commercial and the free-range diet, this is still no absolute number as no information about the ingested amount of feed per day is given. Research on the absolute amount of nutrients ingested should add extra information on top of the reported nutrient concentrations and can correct comparison between nutrient intake of industrial and free-range chickens.

Finally, only two studies about the crop content of non-supplemented fowls are available up to now (Savory et al., 1978; Arshad et al., 2000). Further research into the feed choice and habits of wild

Galliformes can, however, provide important information about their nutrient demand and the ingredients they select to meet those requirements.

Conclusion

Human interference led to less fibre and more protein and energy in the diet of chickens. Based on available literature, the NFE fraction seems not affected, but a more detailed fibre and carbohydrate analysis should evaluate if important differences exist in the contents of enzymatically digestible carbohydrates such as starch. Insects and other invertebrates are found in the majority of free ranging chickens. Seeds rich in non-starch polysaccharides are of low value to the human diet but many appear to be not avoided by chickens, even though correlated with the CF concentration in the diet. The high diet variety shown by this study is an important feature for further exploration when trying to decrease the global feed-food competition.

Chapter 4

Case report: diet and management of free-range chickens in Limpopo, South-Africa

Case report: diet and management of free-range chickens in Limpopo, South-Africa

J. Pauwels, J.W. Ng'ambi and G.P.J. Janssens

Abstract

Although rural chickens are an important source of income and protein for many people on our planet, little information about their management and diet is available. In order to identify ways of increasing the profit for chicken owners, a concrete view about the diet and management of free-ranging chickens is necessary. This trial was performed as a case study in Limpopo, South-Africa. Fifty seven people were questioned about the management of their chickens and the crop content of thirty two chickens was analysed. The results of the questionnaire show that a minimum of money, time and effort is put into the chickens. Both scavenged and supplemented feed is necessary for the chickens to survive. The main reasons for death among the chickens, besides slaughter, are predation and disease. In the chickens' crop, many different and often large ingredients were found. It is estimated, based on numbers both from the questionnaire and literature, that about SAR170 per month could be made by selling the chickens and not changing the management. To improve the profit, the chicken owners should focus on disease prevention and predator protection.

Introduction

Indigenous free-range chickens play an important role in the provision of animal protein, both through meat and eggs, of rural families in developing countries. As the human rural population is estimated to count 3.36 billion heads, which equals 46% of the global population, these chickens should be considered important although their production is low (FAOSTAT, 2015). Exact numbers and accurate information on the management and production of these indigenous chickens is difficult to obtain (Pym et al., 2006). In general, these chickens are kept in traditional free-range systems and their management (or the absence thereof) differs largely from the management that is considered optimal, based on industrial parameters. Improving the profit for rural chicken owners is often focused on the improvement of breeds and diet (Mupeta et al., 2000). Indigenous breeds, however, are naturally selected to fit the rural free-range conditions. As a consequence, they are more resistant to diseases (Minga et al., 2004), care more for their offspring and their agility increases their chances of surviving predators. In addition, their meat and eggs are preferred by the local people (Guèye et al., 1997; Guèye, 1998; Sonaiya et al., 1999). As these rural chickens are from indigenous, slow growing breeds, our study in **Chapter 7** suggested that the quantity of feed, rather than the quality, is the limiting factor for productivity. To determine other ways to increase the profit for rural chicken owners, without tackling the traditional system, the chickens' diet and management was explored. A survey of chicken management was performed among rural chicken owners in Limpopo, South-Africa and the crop content of 32 of their chickens was analysed. Limpopo is the poorest province in the country and nearly one out of four people (23.8% in 2010) have no job (HSRC, 2014). It is therefore suggested that owning chickens can be an important additional source of income and animal protein.

Material and methods

People were questioned in November 2013 across eleven different villages in Limpopo: Xigalo, Tsutsumani, Roadhouse, Mafanele, Gandlanani, Jerome, Basani, Nyavani, Hlanganani, Makhasa and Basopa. The questionnaire was composed in Venda and in English (**Appendix 5**). One hundred and ten people were questioned of which fifty-seven owned chickens and therefore answered the complete questionnaire. Women represented 64% of the chicken owners that were questioned. All hundred and ten people answered the question "why no (more) chickens were purchased". The questions were answered based on a gradual scale. Seven different answers, ranging from “completely disagree” to “completely agree”, from “nothing” to “whole budget” or from “never” to “constantly”, could be chosen. Based on this system, the answers could be regarded as continuous and therefore averages and standard errors could be calculated. Questions on monetary value and time spent were asked to be answered in South-African Rand (SAR 13.8 to EUR 1 for November 2013 (X-rates, 2013)) or in minutes.

From each of the eleven villages the crops of three female chickens were collected when slaughtered for consumption. These chickens were raised on a free-range diet. The owners were asked to leave the chickens outside for scavenging and to not feed them at the day of sampling. The crops were removed immediately after slaughter and stored at -20°C. Crops were defrosted and their contents were macroscopically identified and counted. Due to technical limitations in the field, the nutrient composition of the crop contents was not determined.

Results and discussion

On average, each owner had 8.0 chickens (range 1 to 20) and 11.5 chicks (range 0 to 42). If the households without chickens were included, the average was 4.1 chickens. This is in accordance to the average number of 1 to 10 chickens kept in traditional free-range systems in different developing countries (reviewed by Goromela et al., 2006). More than half of the chicken owners (59%) was unemployed, which was lower compared to the unemployment rate among all people that answered the questionnaire (63%). These numbers, however, largely exceed the reported unemployment rate of Limpopo as reported by HSRC (23.8% in 2010 (HSRC, 2014)) and the overall South-African unemployment rate as reported by the IMF (24.9% in 2013 (IMF, 2015)). The big differences between these numbers could be explained by the moment of questioning, which was during the day, and the specific villages that were questioned.

Within all expenses made on the chickens, feed was indicated to be the most expensive (**Figure 4.1.**). This might explain why chickens are mostly fed kitchen leftovers and, to a lesser extent, grains from own cultivated land. A 1 kg bag of commercial chicken feed was estimated to cost SAR 20 but the owners indicated to rarely feed this to their chickens (**Figure 4.2.**). This might explain why the expenses of feed are not indicated as a reason to limit the flock size (**Figure 4.3.**). An average of 12 minutes a day was spent on the chickens (median: 5 min.; range 1 – 120 min/ day).

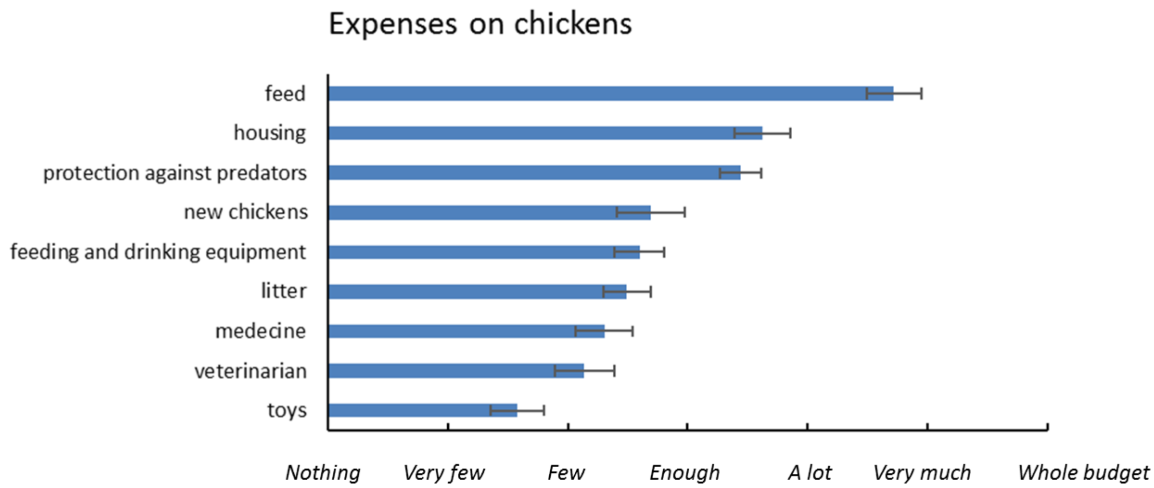

Figure 4.1. Average indication of how much the rural chicken owners (n = 57) spend on different items for their chickens (± st. error).

Reducing kitchen waste was the second most important reason for people to keep chickens, the most important reason was provision of meat for own consumption (**Figure 4.3.**). Eggs for sale or consumption seem to be of less importance, which is, in accordance to the literature, because most eggs are bred by the hens (Pym et al., 2006; Alders and Pym, 2009). Considering that the owners estimate six out of ten chicks to reach the age of six months, breeding the eggs rather than consuming them might be more profitable. Especially when those chickens can be reared on kitchen waste and scavenged feed mainly. The estimated price for an egg was SAR 1.2 and prices for adult hens and roosters were equally estimated at SAR 37 per chicken. As indigenous free-range chickens produce an average of 40 – 60 eggs per year in 3 – 4 clutches (reviewed by Pym et al., 2006), the chicken owners would only make about SAR 100 a year by selling the eggs, assuming that the production would increase when the hens do not sit on their eggs. Based on a an average of six matured hens which produce 40 eggs per year and a survival rate of 60% for the chicks (which is high compared to the literature (Sonaiya et al., 1999)), a 144 chickens per year could be sold when leaving the hens to breed their eggs. At a rate of SAR 37 per chicken and an estimation of 1 kg commercial feed supplementation (SAR 20/ kg) per chicken before they can be sold, roughly SAR 2000 a year or SAR 167 per month could be earned by selling chickens assuming about SAR 450 per year for unexpected expenses. The food poverty line for South-Africa (amount of money that an individual will need to consume the required energy intake) is estimated to be SAR 305 per individual per month.

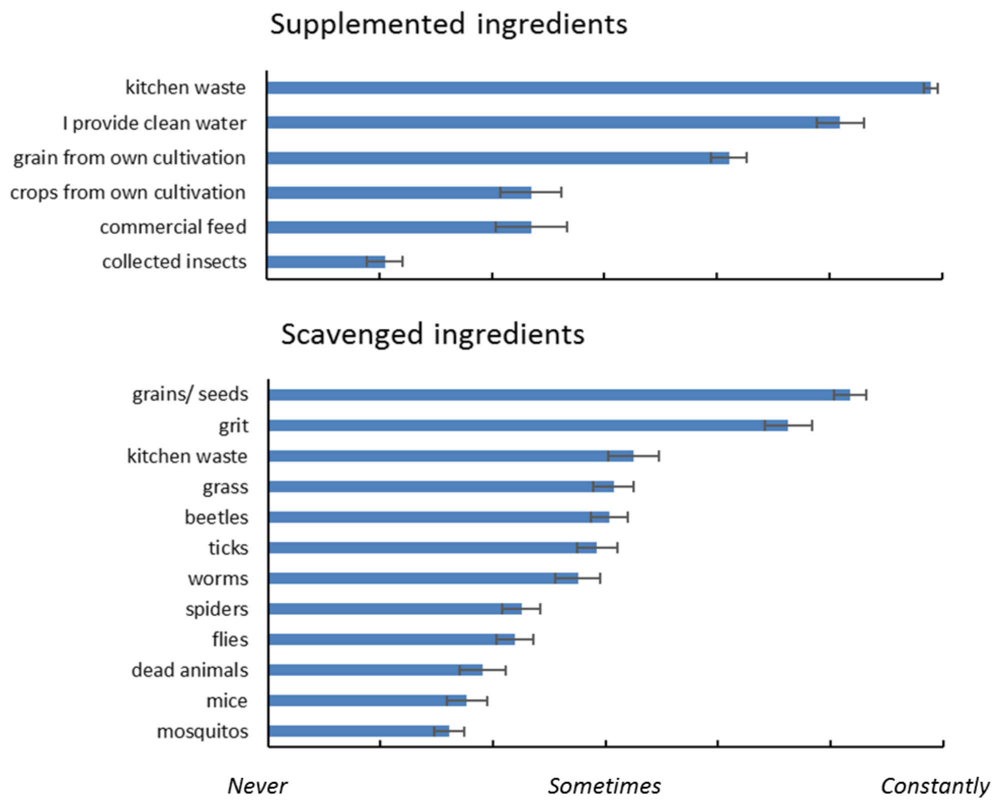

Figure 4.2. Importance, according to the chicken owners, of different ingredients in the chickens' diet. Ingredients, supplemented or from scavenging, are considered (n = 57, ± st. error).

The owners reported that the chickens have access to a lot of space where they scavenge for seeds and grains, grit, grass and insects. This was confirmed by the huge variety of ingredients that was found in the crop content of these chickens. In total, nine different ingredients identified as kitchen waste and nine different invertebrate orders were found (**Table 4.1.**). Moreover, non-nutritional ingredients, such as strings and polystyrene foam were also found and were classified as inert material. Ten out of the thirty-two crops were empty. Only two of the analyzed crops contained corn and no other cereals were found. This in contradiction to **Chapter 3** where cereals are reported to often make up the bulk of the crop content. Because the owners had been asked not to feed the chickens before sampling, the crop content might not fully reflect the situation when the chickens were fed, but might reflect better what the chickens select from real natural feed resources. The owners indicated that both supplemented and scavenged ingredients are needed by the chickens to survive, but, that chickens nearly never die of starvation. The main reasons for chickens to die, besides slaughter, were disease and predation. The most important reason for not keeping (more) chickens was the fact that chicks and chickens often die, which doubts the estimated survival rates (60%) of chicks. The results indicate that, when trying to improve the rural chickens' management, one should focus on animal health and protection against predators. It is, however, not clear whether larger flocks could still be maintained on scavenged feed and kitchen waste supplementation only.

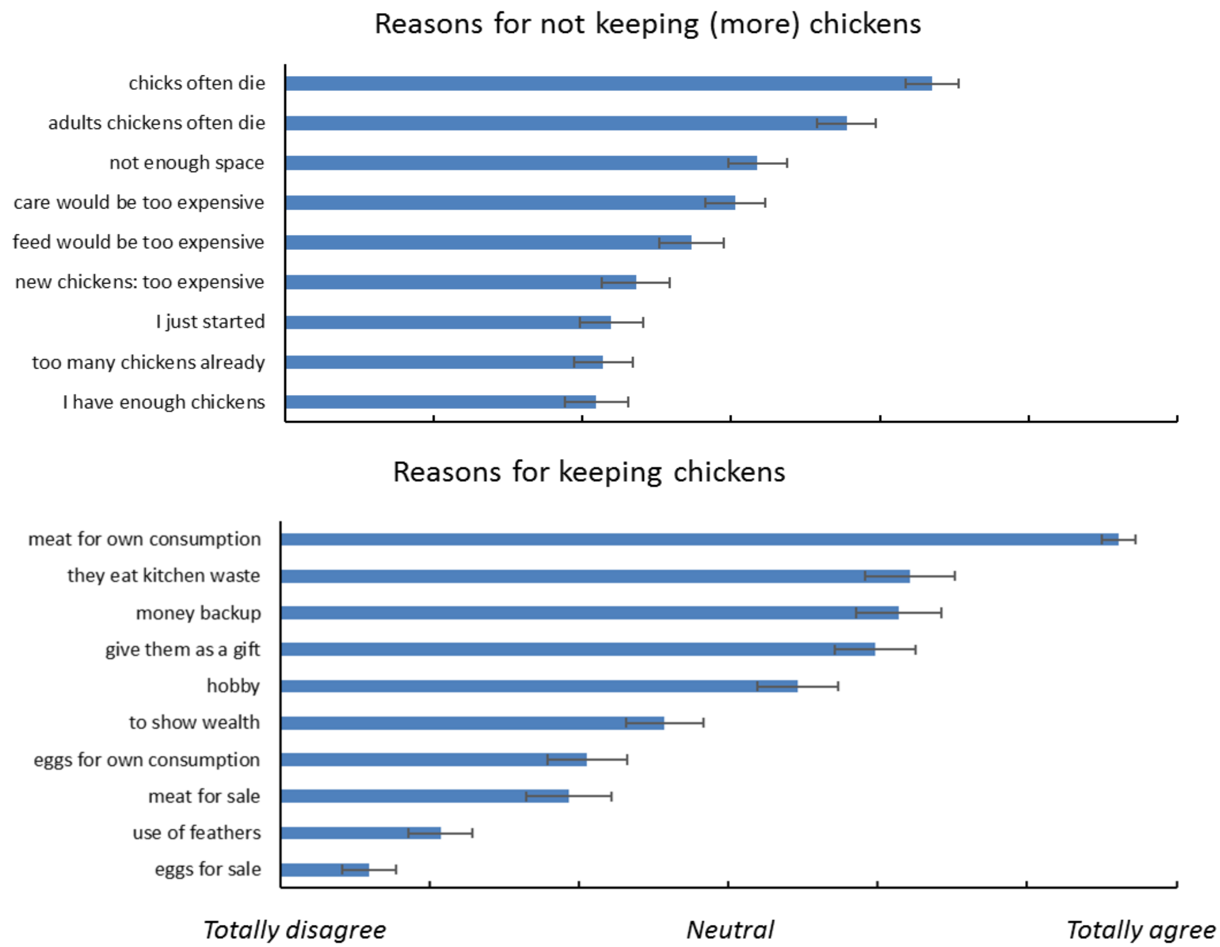

Figure 4.3. Reasons to keep chickens and reasons why not to keep (more) chickens (n = 110 and n = 57, ± st. error).

Table 4.1. Different ingredients and the number of crops (n) in which they were present. Results of macroscopic analysis of crop content of scavenging chickens in Limpopo, South-Africa (n_{total} = 32, 10 empty crops included).

Ingredient	n	Ingredient	n	Ingredient	n
Invertebrate		**kitchen waste**		**plants**	
Ants	13	pap	18	grass	15
small beetles	6	bone fragments	3	fruits	3
Fly	4	egg shells	2	seeds	1
fly larvae	4	tomato peels	2	undefined	7
fly pupae	1	bread	2		
Mosquito	3	fish	2	**grains**	
Millipedes	2	white beans	1	corn	2
Ticks	2	spaghetti	1		
dung beetle	1	meat	1		
sun spider	1				
Cockroach	1	**Inert material**			
Moth	1	hairs	2		
Caterpillar	1	soil	2		
Termites	1	grit	1		
		polystyrene foam	1		
		string	1		

The bodyweight of the average sampled chicken was 1.1 ± 0.3kg. Despite the low bodyweights of some chickens, large items were found in their crops: an entire dung beetle (3.1 cm) in the crop of a 0.8kg chicken and an entire sun spider (2.8 cm) in the crop of a 1.1kg chicken (**Figure 4.4.**). Ticks were found attached to the crop wall.

Figure 4.4. Complete invertebrates found in the crop content of scavenging chickens

Conclusion

It can be concluded that a minimum of time, money and effort is spent on chickens kept in traditional free-range system. The chickens mainly have to provide feed for themselves, which they seem capable of. They, however, need to be supplemented with kitchen waste and grain from own cultivation too. The regular dying of chickens and chicks was the main reason for chicken owners to not expand their flock. As the main causes of death were disease and predation, improving the health and the protection of the chickens should be the focus when trying to improve the management of traditional free-ranging chickens.

Acknowledgements

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Chapter 5a

Cecal drop reflects the chickens' cecal microbiome, fecal drop does not

Cecal drop reflects the chickens' cecal microbiome, fecal drop does not

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Abstract

Microbiota in the gastro-intestinal tract are closely related to both the intestinal and overall health of the host. Experimental chickens have always been euthanized in order to identify and quantify the bacteria in cecal content. In this study, quantification and identification of the microbial populations in cecal drop, cecal content and fecal drop samples from chickens showed that cecal drop contains a bacterial community that is very similar (concerning bacterial diversity, richness and species composition) to cecal content, as opposed to the bacterial community found in fecal drop. Cecal drop analysis thus allows for longitudinal experiments on chickens' cecal bacteria. The varying results in the analysis of fecal samples questions the method's reliability in reflecting the true cecal microbiota in chickens.

Introduction

In the chickens' gastro intestinal tract (GIT) the number and variety of bacteria is highest in the ceca (10^{10} – 10^{11} cells/g) (Barnes et al., 1972; Bjerrum et al., 2006). The cecal microbiome plays an important role in fermentation processes and production of short chain fatty acids (SCFA) (Patterson and Burkholder, 2003; Rehman et al., 2007). Also, the cecum can host pathogenic and zoonotic bacteria that cause severe risks to human health (Herman et al., 2003; Zorman et al., 2006).

Chicken ceca are known to have a complex motility. Several times a day the ceca contract, pushing their content in two directions: towards the ileum and towards the cloaca, excreting a cecal drop (Herrick and Edgar, 1947; Clench, 1999; Janssen et al., 2009). The ceca fill again by use of peristaltic and antiperistaltic contractions at their entrances (Fenna and Boag, 1974). Cecal drops have been studied in chickens before and they were used to detect hazardous bacteria regarding food-safety such as *Campylobacter* or *Salmonella* (Herman et al., 2003; Okamura et al., 2008). An early study (Stern and Robach, 1992) compared three samples: cecal drop, fecal drop and cloacal swab and found cecal drop to be the most sensitive sample for the detection of *Campylobacter*. However, when identifying or quantifying the complete microbiota of the cecum, cecal drop has, to the best of our knowledge, never been used.

In studies with other animal species, the microbiota in one or more parts of the gastro-intestinal tract have been investigated through, for example, excreta, fecal or fistula samples (Harmoinen et al., 2001; De Filippo et al., 2010; Budding et al., 2014). In rabbits, bacteria in the caecotrophes were shown by denaturing gradient gel electrophoresis to be 71% similar to cecal microbiota (Rodriguez-Romero et al., 2009). So far, studies on the entire cecal microbiota of chickens have always been based on samples straight from the cecum, for which chickens had to be euthanized (Bjerrum et al., 2006; Saengkerdsub et al., 2007; Danzeisen et al., 2011).

From a statistical point of view, the required sample size in longitudinal studies will decrease if cecal drop is used, this is because the same birds can be re-sampled for every point in time and differences between individuals will therefore be ruled out. Moreover, from an ethical point of view, the chickens will not need to be euthanized. The aim of this study is to compare the microbiome of three different samples: cecal content, cecal drop and fecal drop and to determine whether these samples can be used as a reference for cecal content and, if so, which of these drops serves as the most effective reference.

Experimental procedures

Experimental setup

Two hundred and forty male chicks, sixty from each of four different breeds (Cobb 500, Cobb-Sasso 175, Sasso and Sussex) were placed in pens of fifteen birds each, with breeds randomly designated to pens. Each pen had a surface of 2 m² and was 75 cm high. The chicks received full vaccinations for Newcastle disease, infectious bronchitis, coccidiosis, Gumboro disease and Marek's disease.

Diets

Two groups of each breed were fed a standard commercial diet and the other two groups were fed an alternate diet containing mealworms (*Tenebrio molitor*), lucerne and ostrich pellets. Both diets were analyzed for dry matter, crude ash, ether extract, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (**Table 5a.1.**). Metabolizable energy (ME) was calculated according to Wiseman (1987). Dry matter and crude ash content were determined by drying the feed to a constant weight at 103°C and combustion at 550°C, respectively. Diethyl ether extract was analyzed using the Soxhlet method (ISO, 1973). Crude fibre was determined using the Association of Official Analytical methods (Association of Official Analytical methods (AOAC), 1995a; Association of official analytical chemists (AOAC), 1995b). To determine NDF and ADF, the methods of Van Soest *et al*. (1991) were used. Crude protein (6.25 × Nitrogen) was determined using the Kjeldahl method (ISO 5983-1, 2005). Water and feed were provided ad libitum. To prevent diarrhea, the chicks fed the alternate diet received a mix of 1/3 alternate diet and 2/3 commercial diet between day 0 and 5. From day 6 to 10 they were fed a mix of 2/3 alternate diet and 1/3 commercial diet. From day 11 on, they were fed the alternate diet only.

Tabel 5a.1. Nutrient composition of the test diets.

	Commercial diet	Alternate diet
Dry Matter (g/ kg OM)	902	911
Crude Ash (g/ kg DM)	56	72
Ether Extract (g/ kg DM)	73	43
Crude Fibre (g/ kg DM)	36	130
Acid Detergent Fibre (g/ kg DM)	13	18
Neutral Detergent Fibre (g/ kg DM)	67	68
Crude Protein (g/ kg DM)	215	187
Metabolizable Energy (MJ)	15	10

OM: Organic Matter, DM: Dry Matter. Metabolizable Energy was calculated according to Wiseman (Wiseman, 1987). All other nutrients were analyzed.

Sampling

Since the chickens were from four different breeds, their growth rates varied. The weekly bodyweight per pen was used to compose a Gompertz curve (GraphPad Prism 5, GraphPad software, USA). Based on the inflection point of this curve, a prediction could be made concerning the point in time that the chickens would achieve their maximum growth rate. In this way chickens were compared at the same physiological age. At the point of maximal growth for a particular breed-diet combination, the chickens from that combination were observed closely. The first cecal drop excreted was taken as a sample immediately after excretion using sterile aliquots and stored in liquid nitrogen. Later (a maximum of 14 minutes), a sample of a freshly excreted fecal drop from the same chicken, was obtained in the same way. Fecal drop was collected from all but two chickens in the designated timeframe. Directly after the collection of both excretions, the chicken was euthanized with an intravenous injection of 1ml sodium-pentobarbital (Release®, 300mg/ml), the GIT was dissected and a sample of cecal content was taken using a sterile aliquot and stored in liquid nitrogen. At the end of the day, the samples were stored at -80°C.

DNA extraction

Bacterial DNA was isolated from each sample using the QIAamp DNA Stool minikit (Qiagen, Venlo, the Netherlands), following the manufacturer's recommendations. The DNA was eluted into DNAse/RNAse-free water and its concentration and purity were evaluated by optical density using the NanoDrop ND-1000 spectrophotometer (Isogen, St-Pieters-Leeuw, Belgium). DNA samples were stored at -20 °C until use in 16S rDNA amplicon pyrosequencing analysis.

16S rDNA gene library construction and pyrosequencing

16S PCR libraries were generated with the primers E9-29 and E514-530, specific for bacteria (Wang and Qian, 2009). The oligonucleotide design included 454 Life Sciences' A or B sequencing titanium adapters (Roche Diagnostics, Vilvoorde, Belgium) and multiplex identifiers (MIDs) fused to the 5' end of each primer. The amplification mix contained 5 U of FastStart high fidelity polymerase (Roche Diagnostics, Vilvoorde, Belgium), 1x enzyme reaction buffer, 200 µM dNTPs (Eurogentec, Liège, Belgium), 0.2 µM of each primer and 100 ng of genomic DNA in a volume of 100 µl. Thermocycling conditions consisted of a denaturation step at 94 °C for 15 min followed by 25 cycles of 94 °C for 40 s, 56 °C for 40 s, 72 °C for 1 min and a final elongation step of 7 min at 72 °C. These amplifications were performed on an Ep Master system gradient apparatus (Eppendorf, Hamburg, Germany). The PCR products were run on a 1% agarose gel electrophoresis and the DNA fragments were extracted and purified using the SV PCR purification kit (Promega Benelux, Leiden, the Netherlands). The quality and quantity of the products were assessed using a Picogreen dsDNA quantitation assay (Isogen, St-Pieters-Leeuw, Belgium). All libraries were run in the same titanium pyrosequencing reaction using Roche MIDs. All amplicons were sequenced using the Roche GS-Junior Genome Sequencer instrument (Roche, Vilvoorde, Belgium).

16S rDNA data processing

The 16S rDNA sequence reads were processed using the MOTHUR software package (Schloss et al., 2009). The quality of all the sequence reads was denoised using the Pyronoise algorithm implemented in MOTHUR and filtered according to the following criteria: minimal length of 425 bp, an exact match to the barcode and 1 mismatch allowed to the proximal primer. The sequences were checked for the presence of chimeric amplifications using Uchime (Edgar et al., 2011). The resultant read sets were compared to a reference dataset of aligned sequences of the corresponding region derived from the

SILVA database of full-length rDNA sequences (http://www.arb-silva.de/) implemented in MOTHUR (Pruesse et al., 2007). The final reads were clustered into operational taxonomic units (OTUs) with the nearest neighbor algorithm using MOTHUR with a 0.03 distance unit cutoff. A taxonomic identity was attributed to each OTU by comparison with the SILVA database (80% homogeneity cutoff). As MOTHUR is not dedicated to taxonomic assignment beyond the genus level, all unique sequences for each OTU were compared to the SILVA dataset, version 111, using the BLASTN algorithm (Altschul et al., 1990). For each OTU, a consensus detailed taxonomic identification was given based upon the identity (less than 1% of mismatches with the aligned sequence) and the metadata associated with the most frequent hits (validated bacterial species or not).

Statistical analysis

Subsampled datasets were obtained and used to evaluate the richness and microbial diversity of the samples using MOTHUR. To capture the multidimensionality of biodiversity, various indices of diversity and community composition were calculated and compared. Rarefaction curves (Colwell and Coddington, 1994), microbial biodiversity (Simpson and non-parametric (NP) Shannon diversity index – (Chao, 2003)), richness estimators (Observed species richness (S_{obs}) and $Chao_1$ estimator – (Chao and Bunge, 2002)) and bacterial evenness were calculated for each sample. Simpson and the NP Shannon index give an estimated index value for diversity. NP Shannon is used when undetected species are present in a sample. Simpson diversity was calculated to measure the probability that two individuals, randomly selected from a sample will belong to the same species. The $Chao_1$ estimator is used to estimate the richness of the detected species (OTUs in this case) in a sample and can be compared to the actual number of OTUs observed in samples. S_{obs} was determined as the number of OTUs present per sample. Evenness was determined to quantify the similarity between samples numerically (Colwell et al., 2012).

Analysis of molecular variance (AMOVA) was performed to compare the genetic diversity between two populations with the genetic diversity that would result from pooling both populations. Additionally, nonmetric multidimensional scaling (NMDS) was used to visualize possible differences in the bacterial communities. The ordination was run in PC Ord (5.0) using the Sørensen distance measure, with six starting dimensions, 40 iterations and an instability criterion of 10^{-5} (McCune and Mefford, 2006). Also

UniFrac was used to calculate distance measures in bacterial communities between sample origins using phylogenetic information (Lozupone and Knight, 2005).

Because three samples were taken from each chicken, we included ‘chicken’ as a random factor in all models to account for pseudoreplication. Moreover, diet and breed could be expected to affect the bacteria in the cecum (Shakouri et al., 2009; Stanley et al., 2012). To analyze the effect of variation by both factors on the difference between the microbiota in cecal content and cecal drop on the one hand and cecal content and fecal drop on the other hand, different mixed models were run. In the first one, cecal content and cecal drop were considered whereas in the second one, only cecal content and fecal drop were taken into account. All first order and two-way interactions between the different variables were tested in a full model. For the final models, the *F* and *P*-values of the explanatory variables were reported in accordance with Murtaugh (Murtaugh, 2014).

Biosample accession numbers

All the biosample sequences were deposited at NCBI (http://www.ncbi.nlm.nih.gov) and are available under the BioProject ID: PRJNA287778.

Results

The day of maximal growth, and therefore the day of sampling, was different for each breed-diet combination (**Table 5a.2.**).

Table 5a.2. Day of maximal growth rate for four chicken breeds fed a commercial or alternate diet

Breed	Cobb		CobbSasso		Sasso		Sussex	
Diet	C	A	C	A	C	A	C	A
Age of sampling	43d	49d	45d	50d	53d	54d	57d	60d

C: commercial diet; A: alternate diet

Across all samples analyzed, a total number of 6667 OTU’s were found, belonging to ten different phyla. *Firmicutes* appeared to be the most abundant phylum in the three samples with a higher level ($P < 0.01$) in fecal drop compared to both cecal content and drop. The second most abundant phylum was the one of the *Bacteroidetes* in cecal content and cecal drop and *Proteobacteria* in fecal drop. *Bacteroidetes* were less abundant ($P < 0.001$) in fecal drop compared to the other two samples. No differences in levels of

Proteobacteria could be found between the three samples. *Actinobacteria* were low in abundance, but still their level was higher ($P < 0.001$) in the fecal samples compared to the cecal samples (**Figure 5a.1.**).

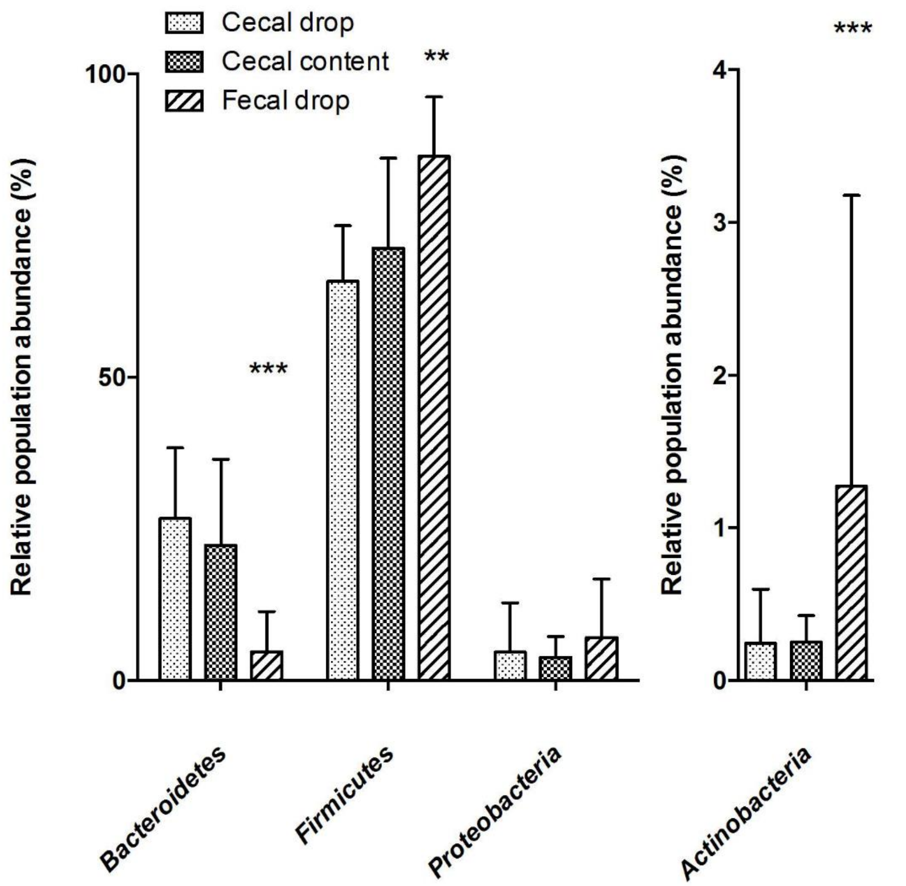

Figure 5a.1. Abundance of the phyla *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* in the three samples: cecal drop, cecal content and fecal drop (with **: $P < 0.01$ and ***: $P < 0.001$). Overall results for the four chicken breeds and both diets, commercial and alternate (n = 14 for cecal drop and cecal content, n = 12 for fecal drop).

Four of the most abundant genera (*Alistipes*, *Bacteroides*, *Lachnospiraceae* and *Ruminococcaceae* unclassified genera) were more abundant ($P < 0.001$; $P < 0.01$; $P < 0.001$ and $P < 0.001$ respectively) for both cecal content and cecal drop compared to fecal drop. In contrast to four other abundant genera (*Enterococcus, Gallibacterium, Peptostreptococcaceae* unclassified genus and *Lactobacillus*) where the level was higher ($P < 0.01$; $P < 0.05$; $P < 0.001$ and $P < 0.001$ respectively) in fecal drop. The abundance estimates of these eight genera did not differ between cecal content and cecal drop (**Figure 5a.2.**). The relative abundance of the most common genera (cut-off is 3%) in the three samples: cecal drop, cecal content and fecal drop are presented (**Figure 5a.3.**).

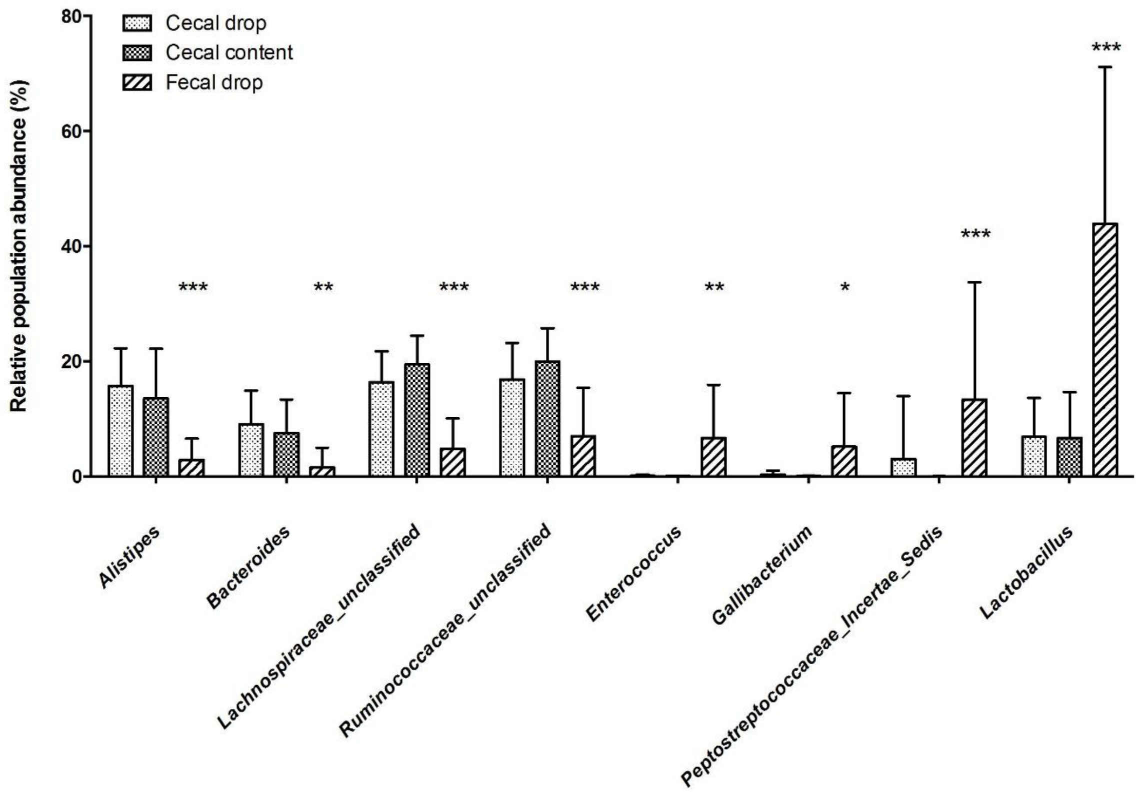

Figure 5a.2. Abundance of bacterial genera in three different samples: cecal drop, cecal content and fecal drop (with *: P < 0.05; **: P < 0.01 and ***: P < 0.001). Overall results for the four chicken breeds and both diets, commercial and alternate (n = 14 for cecal drop and cecal content, n = 12 for fecal drop).

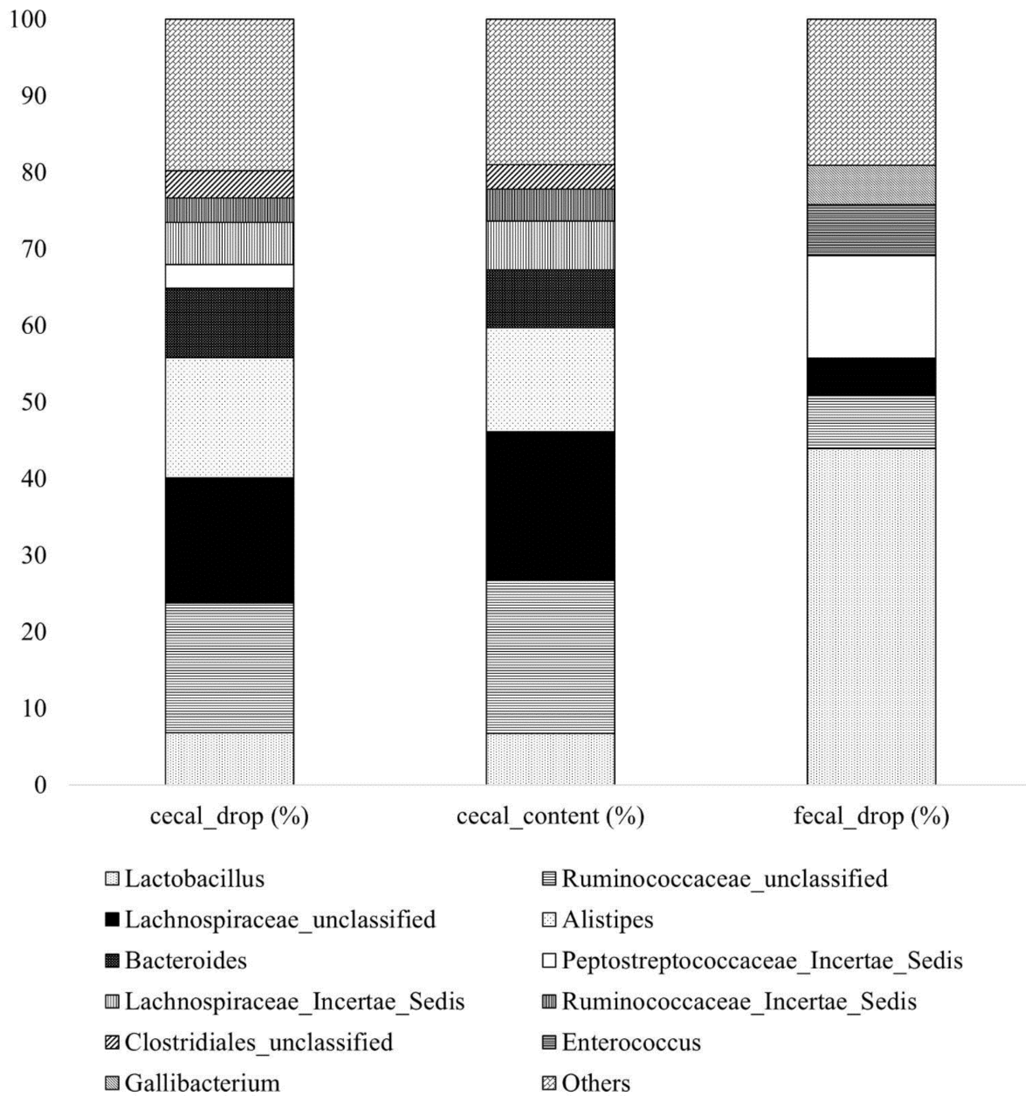

Figure 5a.3. Relative abundance of most common bacterial genera in cecal drop, cecal content and fecal drop (in %). All genera with an abundance > 3% are presented (n = 14 for cecal drop and cecal content, n = 12 for fecal drop).

Diversity

Analysis of molecular variance (AMOVA) indicates a genetic diversity between the bacterial populations found in fecal drop and in cecal content ($P < 0.001$). The same applies for the comparison between fecal drop and cecal drop ($P < 0.001$). The genetic diversity within the bacterial populations found in cecal drop and cecal content did not differ from the genetic diversity when pooling both populations (P = 0.917).

Observed Species Richness (S_{obs}) and $Chao_1$ analysis showed a significantly ($P \leq 0.002$ for both) lower richness in fecal drop compared to cecal drop and cecal content (**Table 5a.3**). Between cecal content and cecal drop, no significant difference in bacterial richness in species level could be found ($P = 0.902$ for S_{obs} and $P = 0.878$ for $Chao_1$).

Bacterial diversity in species level was tested by NP Shannon and Simpson analyses. Both analyses showed a lower ($P \leq 0.001$) diversity in fecal drop compared to cecal drop and cecal content (**Table 5a.3.**). Between cecal content and cecal drop, no significant difference in bacterial diversity in species level (P = 0.805 for NP Shannon and $P = 0.945$ for Simpson) could be found.

Table 5a.3. Mean and standard deviation of the different diversity estimates for three samples: cecal drop, cecal content and fecal drop

	cecal drop	**cecal content**	**fecal drop**	**Sig.**
S_{obs}	260 ± 60 ^{a}	271 ± 46 ^{a}	138 ± 94 ^{b}	< 0.001
$Chao_1$	387 ± 91 ^{a}	407 ± 105 ^{a}	225 ± 133 ^{b}	< 0.001
Simpson	0.06 ± 0.07 ^{a}	0.04 ± 0.02 ^{a}	0.3 ± 0.3 ^{b}	< 0.001
NP Shannon	4 ± 0.7 ^{a}	4 ± 0.3 ^{a}	3 ± 1 ^{b}	< 0.001
Evenness	0.7 ± 0.1 ^{a}	0.8 ± 0.05 ^{a}	0.5 ± 0.2 ^{b}	0.002

Different superscripts (a and b) indicate significant differences using the post hoc Tukey test in the linear mixed model.

Community composition

The Weighted UniFrac of the three samples in species level indicated a similar population structure between cecal drop and cecal content (W-Score: 0.37, $P < 0.001$). The analysis for fecal drop compared to cecal drop and to cecal content showed a higher W-Score (0.85 and 0.89 respectively, $P < 0.001$ for both) indicating a different population structure between fecal drop and both cecal drop and cecal content.

The community composition was further compared with NMDS analysis over two axes since 96% of the variation could be explained by two axes (NMDS1 and NMDS2). For NMDS1 no difference ($P = 0.170$) could be found between cecal content and cecal drop, but both cecal content and cecal drop differed significantly ($P < 0.001$ for both) from fecal drop. The same situation was found for NMDS2, with no difference ($P = 0.497$) between both cecal content and cecal drop and a significant ($P < 0.001$ for both) difference between both cecal samples and fecal drop (**Figure 5a.4.**).

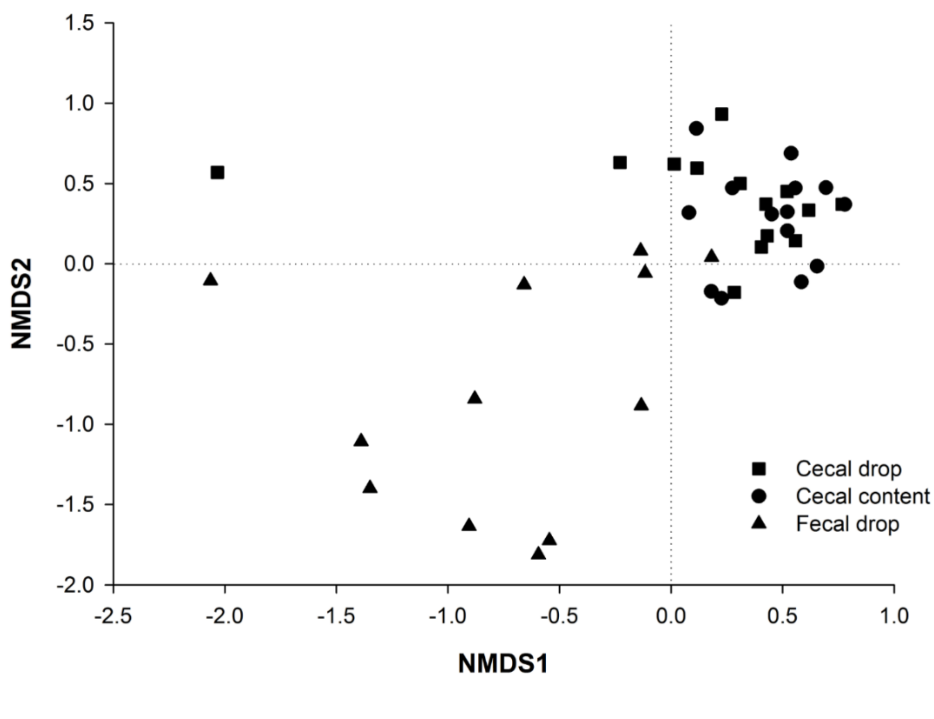

Figure 5a.4. NMDS analysis for three samples: cecal drop, cecal content and fecal drop, over two axes (n = 14 for cecal drop and cecal content, n = 12 for fecal drop).

Variation by breed and diet

Linear mixed models indicated that the similarity between cecal content and cecal drop regarding diversity estimates and community composition was retained when variation was created in the cecal microflora by diet and/or breed. No significant difference in bacterial richness between cecal content and cecal drop was found, neither by S_{obs} nor by $Chao_1$-analysis (P = 0.641 and P = 0.544 respectively). For S_{obs}, no interaction between any of the factors or significant effect by breed or diet was found (P > 0.05). For $Chao_1$, a significant interaction between breed and diet was found (P = 0.015). In addition, Simpson, NP Shannon and Evenness analyses never showed a significant difference between cecal content and cecal drop regarding bacterial diversity (P = 0.305; P = 0.280 and P = 0.218 respectively). The linear mixed models for Simpson, NP Shannon and Evenness, showed no interaction between any of the factors (P > 0.05). Regarding the community composition, no difference between cecal drop and cecal content could be found for NMDS1 or NMDS2 (P = 0.138 and P = 0.102 respectively). Both breed and diet affected the NMDS2 values (P < 0.001 and P = 0.001 respectively), no interactions were found (P > 0.05).

Linear mixed models, analyzing the data concerning cecal content and fecal drop, showed significant interactions between sample and diet for both bacterial richness analyses: S_{obs} and $Chao_1$ (P = 0.008 and P = 0.005). NP Shannon showed a significant interaction (P < 0.001) between sample and diet on bacterial diversity. Breed was not found to have an effect (P > 0.05) on S_{obs}, $Chao_1$ or NP Shannon estimates. For Simpson and Evenness analysis, an interaction between diet and sample (P = 0.004 and P = 0.001 respectively) and breed and sample (P = 0.026 and P = 0.039 respectively) was found regarding bacterial diversity. The means of all diversity and richness estimates indicated a greater diversity for cecal content compared to fecal drop and for the alternate diet compared to the commercial diet. Regarding the community composition, a significant interaction between diet and sample was found for both NMDS1 (P = 0.009) and NMDS2 (P = 0.002). For NMDS2, a significant effect of breed was found (P = 0.004) without interaction with sample or diet.

Discussion

In literature, the use of fecal samples as a reference for the gut microbiota in different species is still under discussion. Three different kinds of studies can be distinguished: the first group of studies consider fecal samples to be a reliable sample in quantifying and identifying the bacteria in the gut (Claesson et al., 2011), the second group limits the use of fecal samples to monitoring shifts in the microbiota of the gut (Mai et al., 2004; Lubbs et al., 2009) and the last group considers fecal samples to be of limited use as a reference for the gut microbiota (Eckburg et al., 2005; Mentula et al., 2005). In our study, fecal drop showed a bacterial diversity, richness and community composition that is low compared to cecal content. This suggests that a fecal sample is not reliable in mapping the complete cecal microbiome in chickens. In addition, the interactions between the factors, sample and diet, were significant for all diversity estimates as well as community estimates, which indicate a different effect of changing diet on the two samples, cecal content and fecal drop. This shows that fecal drop, under the circumstances tested, cannot be considered as a reliable sample to monitor shifts and changes in cecal content.

Cecal drop showed a very similar bacterial diversity and richness and a similar community composition when compared to cecal content. Even when variation by breed and diet was created, no differences could be found in the bacterial community or diversity patterns. These results make cecal drop the best alternative (as a sample unaffected by variation) to monitor the cecal microbiota. This alternative creates an advantage in longitudinal studies since, by use of cecal drop, the same birds can be re-sampled for every point in the time and no correction for individual differences will be required. This will significantly reduce the number of animals needed in trials. In addition, the chickens don't have to be euthanized at the end of the experiment, which will refine the method used in terms of animal welfare. As such, the use of cecal drop analysis will therefore improve two of the three R's (Reduction, Refinement and Replacement) that increase humanity in experiments with animals (Russell and Burch, 1959). In the poultry industry, cecal drop analysis can be used to screen for pathogenic and/ or zoonotic bacteria in the cecum without the need for killing animals to attain cecal content. In addition, this sample will represent the cecal bacteria in a more reliable way than fecal drop analysis.

The alternate diet showed an increase in bacterial diversity and a change in the community composition compared to a standard commercial diet (indicator genera are presented in **Chapter 5b**). It is, however, not clear whether these changes in bacterial diversity and community composition, caused by the diet, also affect functions such as digestive capacity, immunity or gut health.

Cecal content was sampled by separating ileum and cecum and emptying the cecum into an aliquot by squeezing the content from the top of the cecum towards the opening. It must be considered that the sample might, for example, not have included (all of) the mucosa-associated bacteria. Also, by opening the ileocecal junction and squeezing the content out, contact with oxygen could not be avoided. However, this was only for a few seconds, aliquots were closed and stocked in liquid nitrogen immediately. The method of sampling might have resulted in differences in the microbiome in the cecum and the bacteria in the sample of cecal content.

When using cecal drop to map the cecal microbiota, numbers must be considered carefully since facultative anaerobic bacteria tend to overgrow the strict anaerobic bacteria. *Lactobacillus, Peptostreptococcaceae, Enterococcus* and *Gallibacterium* -all facultative anaerobic bacteria, except for some obligate anaerobic *Peptostreptococcaceae*- increased their concentrations when voided as a cecal drop compared to their concentrations in cecal content. However, except for the *Peptostreptococcaceae*, these increases did not change the overall profile. This is in contradiction to the concentrations of the facultative anaerobic bacteria found in fecal drop, being four to seventy times higher compared to that found in cecal content. This could be explained by the longer storage of the feces in the cloaca and by the stickier content of the cecal drop compared to fecal drop, which makes it much more difficult for oxygen to penetrate (Lombardo et al., 1996; Clench, 1999). The concentrations of the strict anaerobic bacteria, *Ruminococcaceae, Lachnospiraceae, Alistipes, Bacteroides* and *Clostridiales* in cecal content compared to cecal drop were very similar. Though, lower concentrations were found in fecal drop for each of them, indicating that they were overgrown.

Conclusion

The bacterial diversity and community composition in fecal drop differs from cecal content for all analyses performed, indicating that fecal drop is an unreliable reference for mapping the cecal microbiota. In addition, the microbiota in fecal drop changed in a different manner compared to the microbiota in cecal content when variation was created by diet and breed. This indicates that fecal drop is not reliable in representing shifts in the cecal microbiome either. Regarding bacterial diversity and community composition, no differences could be found between cecal drop and cecal content, indicating that cecal drop analysis is a good reference for monitoring the microbiota in the cecum. This will reduce the sample size in longitudinal studies considerably and alleviates the necessity to correct for inter-individual differences.

Acknowledgements

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Chapter 5b

Domestication of the chickens' cecal microbiome: commercial *versus* scavenger diet

Domestication of the chickens' cecal microbiome: commercial *versus* scavenger diet

J. Pauwels, M. De Beenhouwer, G.P.J. Janssens and F. Coopman

Abstract

Prebiotic effects are attributed to some fibres (reviewed by Patterson and Burkholder, 2003) and the cecal microbiota in chickens are responsible for the fermentation of fibre (Dunkley et al., 2007). We, therefore, hypothesize that increasing the fibre content in the diet induces a microbial shift, across breeds, in the cecum of chickens and furthermore, increases the presence of some bacterial genera. In this study, the microbial communities in the chickens' cecum are compared between different breeds across two different diets: alternate diet (14.3% crude fibre (**CF**)) and commercial diet (4.0% CF). Ten bacterial genera are found indicative for the alternate diet and two for the commercial diet. No conclusions, however, could be made regarding gut health or digestion.

Introduction

A study on the microbiota of children in Burkina Faso suggested that the gut microbiota coevolved with polysaccharide rich diets, allowing these children not only to maximize energy intake from fibres, but also protecting them from inflammations and noninfectious colonic diseases by production of short-chain fatty acids (**SCFA**) (De Filippo et al., 2010). This was in contrast to European children were the microbiome was less diverse and produced less SCFA. The main cause of these differences was attributed to the different diets as the diet of European children is richer in starch, sugar, calories, fat and animal protein and lower in fibre, compared to the diet of the children in Burkina Faso (De Filippo et al., 2010). As similar differences are observed between the diets of free-ranging and industrial chickens (**Chapter 3**), the question arises whether the microbiome evolved in the same way. The microbiota in the chickens' cecum are known to produce SCFA, as seen in the human colon, by fermentation of the sugars released from non-starch polysaccharides (**NSP**) (Dunkley et al., 2007). These SCFA improve the chickens' gut health by stimulating enterocytes and prevent colonization by pathogenic bacteria such as *Salmonella* (Van Immerseel et al., 2004; Eeckhaut et al., 2008). In this study, we compared the microbial communities in the chickens' cecum from different breeds across two different diets: scavenger diet (14.3% crude fibre (**CF**)) and commercial diet (4.0% CF). We hypothesize that the scavenger diet induces a microbial shift, across breeds, in the cecum of chickens and furthermore, as the scavenger diet is rich in fibres, increases the presence of bacterial genera that are important in the fermentation process of fibres.

Material and methods

For this study all samples of cecal drop and cecal content from **Chapter 5a** were used (n = 28) while fecal drop samples were omitted from this study (n = 12). Consequently, material and methods follows the same methodology as in **Chapter 5a** (Pauwels et al., 2015). Additionally, using PC-ORD 6.0, we ran an indicator species analysis to determine indicator genera for each diet (McCune and Mefford, 2006). This analysis calculated an indicator value based on fidelity and relative abundance of a genus in relation to the diet. By definition, an indicator value of 100 (perfect indicator) implies that the presence of a given genus identifies a diet without error. The obtained indicator values were tested for significance using the Monte Carlo randomization test with 999 permutations.

Results and discussion

The NMDS analysis showed a difference in microbial community composition between the cecal samples of the chickens fed a scavenger diet and the chickens fed a commercial diet. Along the first axis (NMDS1, 54.6% variance explained) a significant difference between both diets was found ($P = 0.046$). For the second axis (NMDS2, 36.2% variance explained) no significant difference was found ($P = 0.37$) (**Figure 5b.1.**). The Indicator Species Analysis found twelve genera to be indicative for a specific diet. Ten of them were indicative of the scavenger diet and two of them were indicative of the commercial diet (**Table 5b.1.**).

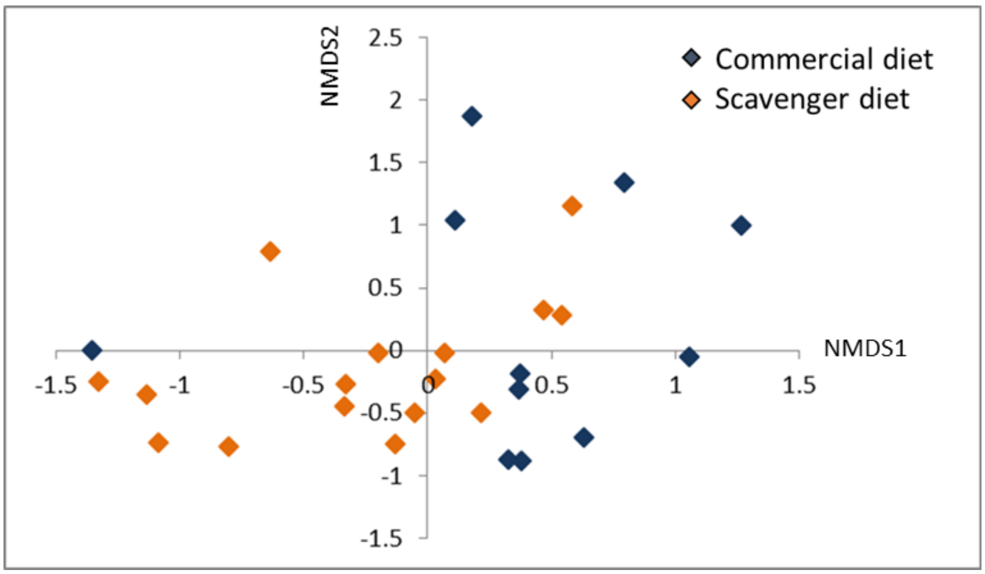

Figure 5b.1. NMDS analysis on two axes, of the community composition of the microflora in cecal drop and cecal content (n = 28) in chickens, displaying the effect of diet. Cummulative $R^2 = 0.91$.

It is difficult to obtain information of the impact of these diet-induced shifts in the cecal microbiota of chickens on the health of the chicken because knowledge of the specific species that change within the genera is too limited and statistically unreliable for now. Many species are “unclassified” or “incertae sedis”, which is in accordance to the literature. Apajalahti et al. (2004), for example, discovered that, based on the microbial DNA, 90% of the species in the gastrointestinal tract of the chicken were previously unknown and more than half of the 640 different species belong to previously unknown genera.

Table 5b.1. Bacterial genera in the chickens' cecum that are indicative of a specific diet.

genus	*Indicator value*	*S. Dev.*	*P*
Genera indicative for scavenger diet			
Campylobacter (*Campylobacteraceae*)	69.3	10.3	0.04
Eggerthella (*Coriobacteriaceae*)	50.3	7.9	0.03
Unclassified (*Coriobacteriaceae*)	81.1	6.6	< 0.001
Unclassified (*Desulfovibrionaceae*)	70.3	6.4	0.01
Incertae Sedis (*Erysipelotrichaceae*)	77.0	6.3	< 0.001
Incertae Sedis, unclassified (Family.XIII.)	68.7	4.3	0.001
Incertae Sedis (*Lachnospiraceae*)	62.8	3.0	0.007
Lactobacillus (*Lactobacillaceae*)	75.0	6.9	0.02
unclassified (RF9)	77.9	5.2	< 0.001
unclassified (*Ruminococcaceae*)	59.0	2.3	0.01
Genera indicative for commercial diet			
Unclassified (*Clostridiales*)	57.6	2.4	0.04
Enterococcus (*Enterococcaceae*)	64.2	9.4	0.02

The higher the indicator value, the higher this genus can be reliably assigned to a specific diet. The *P*-value indicates the goodness of fit of this specific Indicator genus. Only genera with a *P*-value < 0.05 are shown in the table.

Xylan and cellobiose degradation enzymes are similarly found in three main bacterial classes: *Actinobacteria*, *Clostridia* and *Bacteroidia* (Sergeant et al., 2014). Four genera within these classes are indicative for the scavenger diet: *Eggerthella* and an unclassified genus (both within the *Coriobacteriaceae* family), a genus in the *Lachnospiraceae* family and a genus in the *Ruminococcaceae* family. One unclassified genus from the *Clostridiales* family, however, was indicative for the commercial diet.

Although *Campylobacter* is a non-pathogenic bacteria in the cecum of chickens, it is one of the most frequent causes of food poisoning in humans (Humphrey et al., 2007). It is not clear why *Campylobacter* is found as an indicator species for the scavenger diet. Different hygiene and husbandry practices are proven to increase the risk of *Campylobacter* in chickens. Factors related to the care-takers, airflow or water quality are unlikely to cause this difference as both groups were housed together (Refrégier-Petton et al., 2001). Hence, the litter quality of the chickens on the scavenger diet was observed to be more sticky and higher in volume, resulting in dirtier pens.

During fermentation, biochemical pathways produce molecular hydrogen while NADH gets recycled to NAD^+. An accumulation of hydrogen, however, reduces fermentation (Vignais and Billoud, 2007). *Campylobacter*, *Desulfovibrio* spp. (and other sulfate reducing bacteria (Sørensen et al., 1981)), and bacteria from the *Ruminococcaceae* and *Lachnospiraceae* family are potential hydrogen consumers and can, therefore, prevent an impeded fermentation (Sergeant et al., 2014). *Campylobacter* and unclassified or uncertain genera within the *Desulfovibrionaceae, Ruminococcaceae* and *Lachnospiraceae* families are indicator genera for the scavenger diet. It can be expected that a fibre-rich diet increases the fermentation, leading to a hydrogen accumulation. In that situation, bacteria that consume hydrogen will become more important.

Chitin in the diet is known to increase *Lactobacillus* and decrease *E. coli* in the cecum of chickens (Li et al., 2007). As the scavenger diet contained mealworms, it contained chitin too (Richards, 1958). No effect of the scavenger diet on *E. coli* was found which might be due to the relatively low chitin level in the diet (0.1% - 0.5%). Hence, as microbial resistance against *E. coli* is often reported, alternative ways to tackle this bacteria species, for example by adding chitin to the diet, are valuable (Dho-moulin and Fairbrother, 1999). *Lactobacillus* is found indicative to the scavenger diet in this study and is overall considered a beneficial genus as many *Lactobacillus* species are considered probiotics: they produce lactic acid and SCFA and stimulate butyrate-producing bacteria (Dunkley et al., 2007; Meimandipour et al., 2009).

The present design does not answer the question whether either of both diets benefits the health of chickens. In general, both pathogenic and non-pathogenic species can often be included in the same genus. The *Clostridia* genus, for example, is found as an indicator of the commercial diet and is known to include pathogenic and zoonotic species such as *C. perfringens* (Van Immerseel et al., 2004) and non-pathogenic species such as *C. butyricum* (Yang et al., 2010), though this last one can produce botulinum toxins. Another example is the *Campylobacter* genus, indicative of the scavenger diet. A bacterial species such as *Campylobacter jejuni* can be non-pathogenic to the chicken but hazardous to human (Dasti et al., 2010). Moreover, it is important to keep in mind that scavenger diets are extremely variable regarding ingredients and nutrient composition (**Chapter 3**), and, therefore, the results of this study cannot be generalized for all scavenging chickens.

Conclusion

The cecal microbiota in chickens is shown here to differ between chickens, fed on a commercial diet and chickens, fed on a scavenger diet. No differences between the different breeds were found. Ten bacterial genera in the cecal microbiome are indicative for the scavenger diet and two are indicative for the commercial diet. Literature suggests some of them to be important in the fermentation process of fibres. Also, the *Lactobacillus* increase can be explained by the presence of chitin in the diet. These results urge for more studies on the potential health effect of diets that also take into account the natural feeding strategy of chickens.

Chapter 6

Does chitin create a bias in crude protein analysis?

Does chitin create a bias in crude protein analysis?

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Submitted at Food Chemistry

Abstract

The protein content in feed and food is a major determinant of nutritional value. Standard methods, such as Kjeldahl analysis, assume all nitrogen to originate from protein, resulting in a loss of accuracy when non-protein nitrogen is present. In particular, with the increasing use of insects as feed and food ingredient, the nitrogenous non-protein compound chitin is introduced. This study analysed three nitrogen fractions in twenty-three samples: first, total nitrogen was determined according to Kjeldahl analysis; second, nitrogen was determined on the chitin fraction, and third, amino acid nitrogen was calculated based on analysis of amino acids. The results show that correction of total nitrogen level for the nitrogen in chitin improves the accordance with amino acid analysis, which is considered the reference in this study. For pure crab chitin, the conventional Kjeldahl method rendered a concentration of 385 g crude protein/ kg whereas calculations based on amino acids resulted in 3 g protein/ kg only. These results indicate that the protein level in chitin containing diets might be overestimated if not corrected for the nitrogen present in chitin. This correction does not compensate for all non-protein nitrogen sources, but it is a fairly accessible method to obtain a more accurate determination of dietary protein concentration.

Introduction

The value of many diet sources depends on the quality and the quantity of protein. Conventionally, the crude protein concentration in feed and food is determined by the Kjeldahl-analysis (ISO 5983-1, 2005), which is based on the nitrogen (**N**) level in the sample (reviewed by Moore et al., 2010). However, when analyzing feed or food that contains ingredients containing high amounts of non-protein N, crude protein can be overestimated. Analysis of the amino acids gives a clear idea about the real protein content, but these analyses are rather expensive.

Chitin $(C_8H_{13}O_5N)_n$ gets increasing attention as insects become more popular as a protein source for feed and food. As chitin contains 7% of nitrogen (calculated on atomic weights), it might bias the values for crude protein found by Kjeldahl analysis (Weaver et al., 1977). In order to incorporate insects in diets and to recommend them as good protein sources, one must make sure that the analytical methods used are correct and specific, for protein as well as for chitin.

Methods based on fibre analysis have been used to determine chitin in samples (Finke, 2007; Liu et al., 2012). However, these methods are not applicable to determine chitin in a diet that is composed of both animal and plant derived materials, as crude fibre might give an overestimation of chitin with these tests. The aim of this study was to evaluate a method to improve the accuracy of protein and chitin determination in diets. Therefore, three nitrogen fractions were analysed in twenty-three samples: first, total nitrogen was determined according to Kjeldahl analysis; second, nitrogen was determined on the chitin fraction, and third, amino acid nitrogen was calculated based on analysis of amino acids. The difference between total nitrogen and the two others was defined as non-protein non-chitin nitrogen. Total nitrogen ($N_{Kjeldahl}$) was split in three sections depending on their origin: protein (N_{aa}), chitin (N_{chitin}) and others such as DNA and some vitamins (folic acid and thiamine for example56) (N_{npnc}) (**Figure 6.1.**).

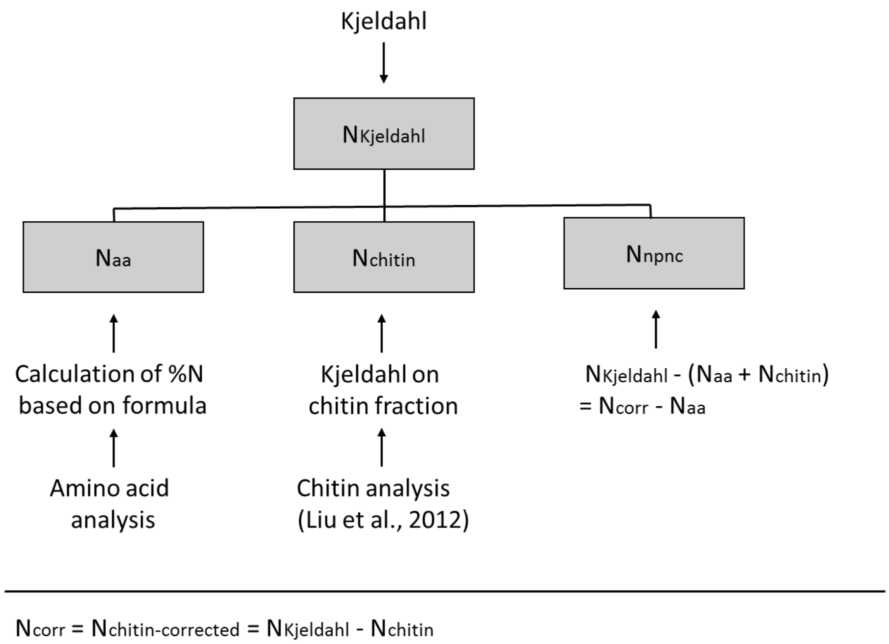

Figure 6.1. The different nitrogen (N) fractions ($N_{chitin\text{-}orrected}$, N_{chitin}, $N_{amino\ acid}$ and N_{npnc}) were determined for each sample.

Material and Methods

Samples

In total, twenty-three different samples were analysed. Ten were from insect origin: *Tenebrio molitor* adult (fed wheat bran only) (Ecology Projects®), *Tenebrio molitor* larvae (fed wheat bran only and fasted for ten days) (Ecology Projects®), *Tenebrio molitor* larvae (fed wheat bran and oats, not fasted) (Ecology Projects®), *Tenebrio molitor* pupae (fed wheat bran only) (Ecology Projects®), *Hermetia illucens* adult and *Hermetia illucens* pupae (fed mixed kitchen waste), *Locusta migratoria migratorioides* instar 5-6 and *Locusta migratoria migratorioides* adult (Ecology Projects®), *Galleria mellonella* larvae (parboiled) (Ecology Projects®) and *Alphitobius diaperinus* larvae (Ecology Projects®); two were from crustacean origin: *Gammarus pulex* (Versele-Laga®), *Macrobranchium nipponense* (Versele-Laga®); three low protein ingredients: chitin from shrimp shells (Sigma®), cellulose powder from spruce (Fluka®) and wood shavings (Roose®); and eight protein containing ingredients: casein from bovine milk (Sigma®), feathermeal, wheat bran (Lannoo paardenvoeders®), soy hulls (Lannoo paardenvoeders®), instant yeast (*Saccharomyces cerevisae*) (Bruggeman®), dried mushrooms (*Boletus edulis*, le Montagnard®), porcine collagen (Vepro®) and beef collagen (Federal Laboratories Corporation®).

Analyses

All samples were dried and ground (screen size 1mm). Diethyl ether extract was removed from the samples by use of the Soxhlet method (ISO, 1973). Subsequently, the samples were loaded in filter bags (F57, Ankom Technology®), using two bags per sample with one gram of sample per filter bag and sealed by use of Vacopack F380 (KRUPS®). Chitin was determined after digestion with HCl and NaOH of the filter bags, based on Liu *et al.,* (2012). Blank filter bags were analysed similarly and those results were used to correct N_{chitin} of the samples. Subsequently, the nitrogen level in the analysed chitin (**N_{chitin}**) was determined by Kjeldahl, assayed according to the Association of Official Analytical Chemists methods, method 934.01 (AOAC, 2006). For this analysis, the samples remained in the filter bags. Total nitrogen (**$N_{Kjeldahl}$**) was determined on each sample by standard Kjeldahl analysis (AOAC method 934.01). The insoluble ash content was determined using the procedure of Van Keulen and Young (1977), as adapted by Atkinson et al. (1984) and was used to correct the N_{chitin} content for residual ash. The calculated chitin content was determined using the conversion factor 14.5 (based on the atom weights (**A**) in chitin $(C_8H_{13}O_5N)_n$: $(A_N/ (8 \times A_C + 13 \times A_H + 5 \times A_O + 1 \times A_N))^{-1}$ (with $A_N = 14$, $A_C = 12$, $A_H = 1$ and $A_O = 16$).

The amino acid content of each sample was determined by use of the amino acid analyser (Sykam, Eresing, Germany) including the oxidation step as described by the International Organisation for Standardization (ISO 13903, 2005). This method does not give reliable results for Trp and for Phe content in chitin, therefore, Trp was not taken into account for all samples and Phe was not taken into account for chitin. Total protein nitrogen (**N_{aa}**) was calculated based on the formula of each amino acid and the results of the amino acid analysis per sample. Amino acid levels below the detection limit (< 0.1 g/ kg) were considered zero.

Calculations

Chitin was calculated as $N_{chitin} \times 14.5$. The corrected level of N for chitin (**N_{corr}**) was calculated as $N_{Kjeldahl} - N_{chitin}$ and the non-protein non-chitin N (**N_{npnc}**) was calculated as $N_{Kjeldahl} - N_{chitin} - N_{aa}$. $Protein_{aa}$ is calculated as the sum of all individually determined amino acids and $Protein_{crude}$ is calculated as $N_{Kjeldahl} \times 6.25$. Linear relationships between N_{aa}, N_{corr}, and $N_{Kjeldahl}$ and $Protein_{aa}$, $Protein_{crude}$ and $Protein_{corr}$ were calculated by linear regression analysis using SPSS Statistics 23.

Results

Terrestrial vertebrate derived ingredients had the highest $N_{Kjeldahl}$, followed by the insects and crustaceans. Mushroom, chitin and yeast had intermediate $N_{Kjeldahl}$ concentrations, plant derived ingredients were the second lowest and cellulose and wood shavings contained nearly no $N_{Kjeldahl}$ (**Table 6.1.**).

Pure chitin, derived from shrimp shells, had a N_{chitin} content of 55 g/ kg, therefore, 797.5 g calculated chitin per kg was found which was lower than the analysed chitin (973 g/ kg). Porcine collagen, soy hulls and wood shavings showed high levels of analysed chitin and low levels of calculated chitin. For casein, bovine collagen and wheat bran, slightly negative values were found for N_{chitin} and, therefore, their calculated chitin levels were negative as well. Negative values for analysed chitin were found for cellulose, feathermeal and casein (**Table 6.1.**).

Table 6.1. Different nitrogen (N) fractions (N_{chitin}, N_{corr}, N_{aa} and N_{npnc}) of the total N ($N_{Kjeldahl}$) in feed samples of different origin: chitin, insects, crustaceans, plants, fungi and terrestrial vertebrates (g/ kg). Chitin content in different samples were both analysed and calculated (g/ kg).

	N Kjeldahl	N chitin	N corr	N aa	N npnc	Analyzed chitin	Calculated chitin
Chitine	61.6	55.0	6.6	0.5	6.1	973	797.5
Insects							
Tenebrio molitor (adult)	117.4	8.3	109.1	68.2	40.9	163	120.4
Tenebrio molitor (larvae, fasted)	81.6	2.3	79.3	53.8	25.5	117	33.4
Tenebrio molitor (larvae, not fasted)	97.9	5.2	92.7	57.3	35.4	88	75.4
Tenebrio molitor (pupae)	97.1	10.0	87.1	60.6	26.5	69	145.0
Locusta migratoria (instar 5-6)	98.2	7.0	91.2	56.0	35.2	134	101.5
Locusta migratoria (adult)	96.8	8.7	88.1	53.6	34.5	157	126.2
Hermetia illucens (adult)	84.5	5.1	79.4	61.7	17.7	87	74.0
Hermetia illucens (pupae)	76.2	18.0	58.2	46.3	11.9	239	261.0
Galleria mellonella (larvae, parboiled)	59.8	3.0	56.8	40.8	16.0	53	43.5
Alphitobius laevigatua (larvae)	85.0	6.8	78.2	64.0	14.2	96	98.6
Crustacea							
Macrobranchium nipponense	100.3	1.6	98.7	72.3	26.4	15	23.2
Gammarus pulex	79.2	2.9	76.3	57.2	19.1	39	42.1
plant and fungal derived ingredients							
Cellulose	7.0	0.0	7.0	0.1	6.9	-2	0.0
Wood shavings	7.1	3.2	3.9	0.9	3.0	575	46.4
Soy hulls	23.2	3.8	19.4	12.9	6.5	221	55.1
Wheat bran	31.0	-0.3	31.3	19.1	12.2	50	-4.4
Mushroom	52.5	4.6	47.9	24.9	23.0	84	66.7
Yeast	71.7	1.7	70.0	52.6	17.4	49	24.7
terrestrial vertebrate derived							
Casein	139.8	-0.4	140.2	119.5	20.7	-8	-5.8
Collagene bovine	158.6	-0.8	159.4	138.4	21.0	4	-11.6
Collagene porcine	145.3	1.4	143.9	107.7	36.2	230	20.3
Feathermeal	143.4	0.4	143.0	117.7	25.2	-6	5.8

With $N_{Kjeldahl}$ determined by Kjeldahl analysis on complete sample; N_{chitin} determined by Kjeldahl analysis on chitin fraction; N_{corr} calculated as $N_{Kjeldahl} - N_{chitin}$; N_{aa} calculated on molecular weights and amino acid content of each sample; N_{npnc} (non-protein non-chitin nitrogen) calculated as $N_{Kjeldahl} - (N_{chitin} + N_{aa})$; Analysed chitin was determined according to Liu *et al.* (2012); Calculated chitin was calculated as $N_{chitin} \times 14.5$.

Table 6.2. $N_{Kjeldahl}$ (g/ kg), $Protein_{Kjeldahl}$ (g/ kg), $Protein_{aa}$ (g/ kg) and $Protein_{aa}$/ N_{aa} for each sample.

	N Kjeldahl	Protein Kjeldahl	Protein aa	Protein aa/ Naa
Chitine	61.6	385	3.2	6.46
Insects				
Tenebrio molitor (adult)	117.4	734	433.3	6.36
Tenebrio molitor (larvae, fasted)	81.6	510	352.8	6.55
Tenebrio molitor (larvae, not fasted)	97.9	612	368.0	6.42
Tenebrio molitor (pupae)	97.1	607	387.4	6.40
Locusta migratoria (instar 5-6)	98.2	614	337.7	6.03
Locusta migratoria (adult)	96.8	605	352.4	6.58
Hermetia illucens (adult)	84.5	528	393.9	6.38
Hermetia illucens (pupae)	76.2	476	256.7	5.55
Galleria mellonella (larvae, parboiled)	59.8	374	267.9	6.57
Alphitobius laevigatua (larvae)	85	531	413.7	6.46
Crustacea				
Macrobranchium nipponense	100.3	627	471.3	6.52
Gammarus pulex	79.2	495	361.7	6.32
plant and fungal derived ingredients				
Cellulose	7	44	0.5	5.83
Wood shavings	7.1	44	6.3	6.98
Soy hulls	23.2	145	83.6	6.50
Wheat bran	31	194	128.2	6.70
Mushroom	52.5	328	154.3	6.19
Yeast	71.7	448	359.2	6.83
terrestrial vertebrate derived				
Casein	139.8	874	851.0	7.12
Collagene bovine	158.56	991	861.1	6.22
Collagene porcine	145.28	908	670.0	6.22
Feathermeal	143.36	896	805.2	6.84

$N_{Kjeldahl}$ determined by Kjeldahl analysis on complete sample, $Protein_{Kjeldahl}$ (g/ kg) (calculated as $N_{Kjeldahl} \times 6.25$) represents the classical calculation of crude protein, $Protein_{aa}$ (g/ kg) is the sum of amino acids and $Protein_{aa}$/ N_{aa} is the estimation of the specific nitrogen:protein conversion factor.

The linear relations found between $N_{Kjeldahl}$, N_{corr} and N_{aa} can be described as (**Figure 6.2.**):

$$N_{Kjeldahl} = 24.76 + 1.05\ N_{aa}\ (R^2 = 0.894;\ P < 0.001)$$

$$N_{corr} = 12.18 + 1.16\ N_{aa}\ (R^2 = 0.960;\ P < 0.001)$$

Linear relations found between $Protein_{aa}$ and $Protein_{crude}$ on the one hand and $Protein_{aa}$ and $Protein_{corr}$ on the other hand (**Figure 6.3.**):

$$Protein_{crude} = 167 + 0.98\ Protein_{aa}\ (R^2 = 0.872;\ P < 0.001)$$

$$Protein_{corr} = 88.3 + 1.08\ Protein_{aa}\ (R^2 = 0.944;\ P < 0.001)$$

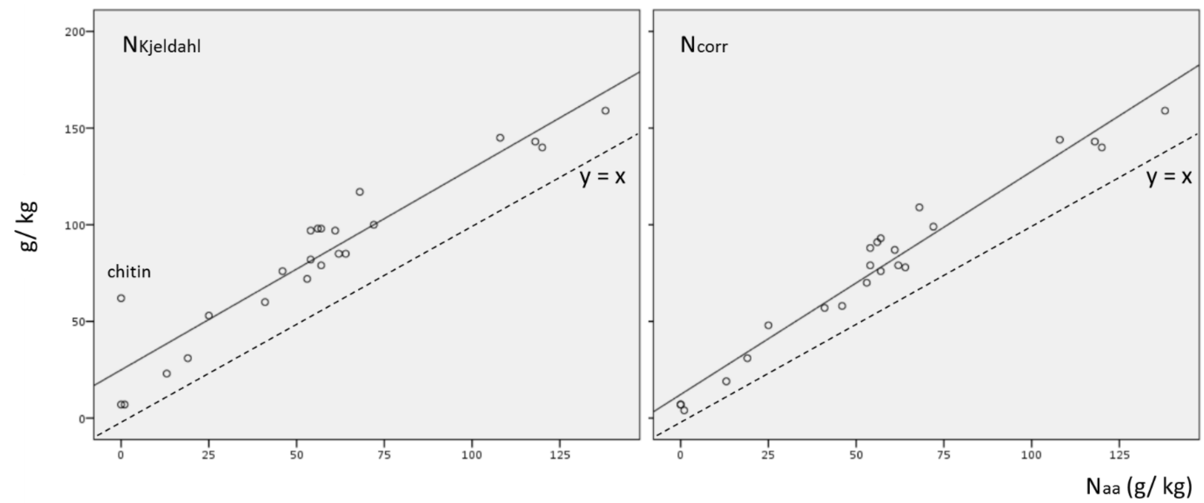

Figure 6.2. Linear relation between $N_{Kjeldahl}$ and N_{aa} and between N_{corr} and N_{aa} (n = 23)

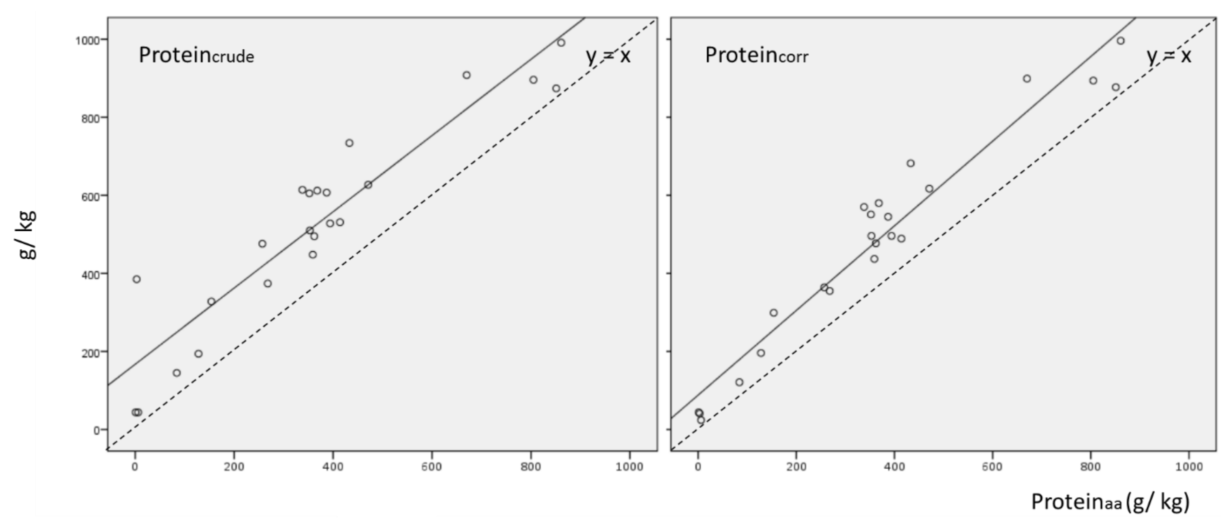

Figure 6.3. Linear relation between $Protein_{aa}$ and $Protein_{crude}$ and between $Protein_{aa}$ and $Protein_{corr}$ (n = 23)

Discussion

Samples were compared on N level as conversion factors for calculating crude protein are very sample specific (Boisen et al., 1987; Mariotti et al., 2008). Nitrogen, calculated on the amino acid composition of the different samples (N_{aa}), is considered as the reference. However, the amino acid analysis is very destructive (ISO 13903, 2005) and could result in a slight underestimation of the true amino acid content and, therefore, of the N_{aa}. In contrast, N_{corr} might show a slight overestimation as it is calculated as $N_{Kjeldahl}$ - N_{chitin} and therefore still includes non-protein non-chitin sources of nitrogen (N_{npnc}), such as DNA and amides.

Another reason for the crude protein to overestimate true protein, is the use of the conversion factor 6.25, which is based on maize (Jones, 1931). Conversion factors for the samples analysed in this study can be estimated as $Protein_{aa}$/ N_{aa}. Conversion factors found in literature, however, often differ largely from these results. For example: soy hulls (5.4), wheat bran (6.31), mushrooms (3.99), casein (6.38), collagens (5.55) and feathermeal (6.21) were found in literature (Jones, 1931; Dintzis et al., 1979; Fujihara et al., 1995; Tiwary and Gupta, 2012). It would be interesting to review the variation on nitrogen: protein conversion factors for different samples.

Regarding the linear relations with N_{aa} and $Protein_{aa}$, R^2 was bigger for the corrected values (N_{corr} and $Protein_{corr}$, respectively) compared to $N_{Kjeldahl}$ and $Protein_{crude}$, respectively. This indicates that estimating N_{aa} and $Protein_{aa}$ is most reliable when using N_{corr} and $Protein_{corr}$.

Within the same species, N_{chitin} is highest for the pupae and lowest for the larval stages, with the adult stages intermediate. This is in line with Bukkens' suggestions that the chitin level is lower when the body of the insect is softer, but is contradicted by Finke (Bukkens, 1997; Finke, 2007). For pure chitin, the method of Liu et al. (2012) gave accurate results (973 g/ kg), although the calculations based on N_{chitin} and conversion factor 6.90 (A_N/ $(5 \times A_O + 13 \times A_H + 8 \times A_C + A_N)^{-1}$ based on the formula for chitin ($(C_8H_{13}O_5N)_n$) gave a lower result (807 g/ kg). Different sources of chitin exist and the chemical compositions might change slightly depending on the origin of the chitin (Tshinyangu and Hennebert, 1996). When calculating the nitrogen: chitin conversion factor from our results (N_{chitin}/ analysed chitin = 55/ 973 (both in g/ kg)), a conversion factor for chitin from crab shells of 5.65 is found.

High values of analysed chitin for soy hulls, wheat bran and wood shavings were found, though, these ingredients are generally accepted to contain no chitin. Comparing these values with those for calculated chitin shows that low levels of N were present in the analysed chitin fraction. As the method of Liu et al. (2012) is close to the methods to analyse crude fibre (DeVries, 2003; Finke, 2007), and crude fibre does not contain nitrogen, it can be assumed that crude fibre induced an overestimation for the chitin analysis.

$Protein_{Kjeldahl}$ overestimates the protein content in the samples analysed in this study. This overestimation is both due to the lack of an exact conversion factor for each sample (Boisen et al., 1987; Mariotti et al., 2008) and the overestimation of protein nitrogen. The bias of N_{chitin} in the protein calculation is clear when looking at pure chitin. $N_{Kjeldahl}$ of pure chitin is 62 g/ kg, resulting in an estimation of 385 g/ kg $Protein_{crude}$. Hosain and Blair (2007) found a similar crude protein content for chitin (373 g/ kg) (Hossain and Blair, 2007). Estimating the true protein content by N_{corr} ($N_{Kjeldahl}$ – N_{chitin}) is perfect neither as sources such as amides and DNA still contribute. Although, N_{corr} is more correct to estimate the protein content than $N_{Kjeldahl}$. In insects, N_{chitin} rarely exceeds 10g/ kg and, therefore, the overestimation of protein in insects is rarely bigger than the conversion factor (Finke, 2007). Still, it is valuable to know N_{chitin}. On the one hand, one can use N_{chitin} to distinguish between chitin and fibre when interpreting the results of the chitin analysis. On the other hand, when chitin is included in high doses, the correction of $N_{Kjeldahl}$ for N_{chitin} will be important.

Conclusion

The specificity for the crude protein and the chitin analysis strongly depends on the type of feedstuff. The crude protein analysis will overestimate the protein content in all samples containing chitin: insects, crustaceans and fungi, though this overestimation might be small. Standard Kjeldahl analysis showed a crude protein level of 38.5% in pure chitin. Correcting the nitrogen level for the nitrogen level in chitin, gives values closer to the nitrogen level in the amino acids. However, this correction does not include the nitrogen derived from non-protein non-chitin sources such as amides and DNA. Sample specific conversion factors are needed for each sample.

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Chapter 7

Selection for growth performance in broiler chickens associates with less diet flexibility

Selection for growth performance in broiler chickens associates with less diet flexibility

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Abstract

Global competition for high standard feed-food resources between man and livestock, such as industrial broilers, is a concerning problem. In addition, the low productivity of scavenger chickens in developing countries leaves much to be desired. Changing the ingredients, and therefore, the nutrient composition of feed intake by commercially fed as well as scavenger chickens seems like an obvious solution. In this study, the ability of four broiler chicken breeds to perform on a commercial versus a scavenger diet was tested. The four broiler breeds differed genetically in growth potential. A significant ($P < 0.01$) negative effect of the scavenger diet on the bodyweight of the fast growing breeds was found and this effect decreased with decreasing growth rate in the other breeds. These differences in bodyweight gain could not be explained by differences in digestive capacity but were caused by the lack of ability of the fast growing breeds to increase their feed intake sufficiently.

Introduction

The feeding of industrial broiler chickens is often criticized because of the extensive use of feed sources which are neither socially nor ecologically sustainable (Delgado et al., 2001; Barona et al., 2010; Wilkinson, 2011; Erb et al., 2012). The diet of intensively-raised broilers consists mainly of maize, soy and wheat, ingredients that could also be used directly in the human diet (Donohue and Cunningham, 2009; Foley et al., 2011; Wilkinson, 2011). Ravindran (Ravindran, 2011) and Farrell (Farrell, 2005) proposed alternative ingredients, less sought after in the human diet, that could be used in the chickens' diet.

The low productivity of scavenger chickens in developing countries is often blamed on the lack of concentrated diets (Farrell, 2005; Badubi et al., 2006). The diet of scavenger chickens contain, for example, crude fiber levels up to more than 100g/kg dry matter (Dessie and Ogle, 2001). While Jørgensen *et al.* (1996) reported that broilers (by fermentation of three different NSP rich substrates: pea fibre, wheat bran and oat bran) extract a maximum of 42kJ/d, representing only 3 to 4% of the daily metabolisable energy intake, hereby confirming the widely accepted idea about the low energetic value of NSP-rich diets for poultry (Moran, 2006).

Solving both problems, both for the industrial as for the rural chickens, is especially interesting as chickens are a widespread food source around the globe. The FAO estimated that there were nearly 22 billion living chickens in 2012 (FAOSTAT, 2015). This is the equivalent of more than three chickens per person. Moreover, in developing countries, chickens are often the main source of animal protein through their meat and eggs and most of these chickens stem from indigenous, slow growing, breeds (Neumann et al., 2002; Sonaiya, 2007).

In this study four different chicken breeds, with growth rates between 30 and 60 g/ d, were used to plot a range in growth rate between the slow growing scavenger chicken on one side and the fast growing industrial broiler chicken. Two diets were used to feed these chickens, one was a commercial industrial diet while the other was based on reported scavenger diets (Dessie and Ogle, 2001; Goromela et al., 2008; Mekonnen et al., 2009; Momoh et al., 2010). The average daily bodyweight gain, average daily feed intake, feed conversion ratio and the length of the *tarsometatarsus* were registered for all breeds on each of the diets. The aim of this study was to monitor the effect of the scavenger diet in relation to breed-specific growth rate.

Materials and Methods

Animals and housing

Four different broiler breeds were selected based on their commercial growth rate from hatching weight to slaughter weight (hereafter referred to as breed-specific growth rate): Cobb 500 (60 g/d) (Cobb-Vantress, 2012), Cobb-Sasso 175 (46g/d) (Sasso, 2010a), Sasso (XL44 × SA_{51}(A)) (38g/d) (Sasso, 2010b) and Sussex (Sussex × SA_{51}(A)) (30g/d) (Sasso, 2010c). A total of 240 one day old male chicks, sixty from each breed, were obtained from the same hatchery (‘t Gulden Ei, Kruishoutem, Belgium). From each breed, 15 chicks were randomly assigned to 4 pens littered with wood shavings. Each pen contained 15 animals and breeds were not mixed. Each pen had a surface of $2m^2$ and was 75cm high (conform ETS 123 as birds were at all times < 2.4 kg). During the first week, 23 hours of light were provided per day and from day 8 the light period was reduced to 18 hours per day. At hatching all chicks were vaccinated against Newcastle disease (Nobilis, NDC2®), infectious bronchitis (Nobilis, IB MA5®) and Marek’s disease (Fort Dodge, Poulvac Marek HVT®). Four day old chicks were vaccinated against coccidiosis (Intervet, Paracox-5®). At 19 days the vaccination against Newcastle disease (Nobilis, ND Clone 30®) was repeated and a vaccination against Gumboro (Nobilis, Gumboro D78®) disease was performed at 22 and 25 days.

Diets and Treatments

From hatching day (day 0), two pens of each breed were fed the commercial diet and two pens from each breed were fed the scavenger diet. A representative sample from eight randomly chosen feed bags of each diet was taken and pooled for both diets. Both samples were analysed in duplicate for nutritional values (**Table 7.1.**). The commercial diet (Fini Pur Croc®, Versele-Laga, Deinze, Belgium) was mainly composed of wheat (42%), soybean meal (22%), maize (10%) and maize middlings (5%) and it contained two enzymes: 6-phytase and E1617-Endo-1,4-β-xylanase. This diet was ground into flour and 10 g/ kg of Celite® (VWR International, Leuven, Belgium) was added as source of acid insoluble ash (**AIA**) in order to determine diet digestibility (Scott and Hall, 1998). The scavenger diet contained 930 g/kg Austru 2 Growth® (wheat (20%), sunflower meal (20%), maize (12%), wheat gluten (11%) and wheat semolina (11%), soybean meal (6%) and spelt bran (5%)) (Versele-Laga, Deinze, Belgium), 40g/kg dried mealworms (*Tenebrio molitor*), 20g/kg dried lucerne and 10g/kg Celite®. All components were ground into flour and mixed until a homogenous feed was acquired. The Austru 2 Growth® contained no additional enzymes. To accustom the animals to the scavenger diet, groups fed this diet received a mixture of one third scavenger diet and two thirds commercial diet from day 0 to 5, two thirds scavenger diet and one third commercial diet from day 6 to 10, and from day 11 onwards only the scavenger diets was offered. Drinking water was provided ad libitum in drinking cups.

Table 7.1. Nutrient and energy concentration of both test diets: commercial and scavenger diet

	Commercial diet	*Scavenger diet*
Dry matter (g/kg)	902	911
Crude protein (g/kg)	215	187
Ether extract (g/kg)	81	47
Crude fibre (g/kg)	40	143
Acid detergent fibre (g/kg)	13	18
Neutral detergent fibre (g/kg)	67	68
Ash (g/kg)	62	79
Acid-insoluble ash (g/kg)	15	15
Metabolisable energy (MJ/kg)	15	10

Measurements and sampling

Starting from day 0, the chickens were weighed weekly until day 36 and the average bodyweight per pen was calculated. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) were calculated over the period of 36 days and were corrected for bodyweight at hatching and mortality during the trial. To collect excreta for the digestibility trials, a container (1cm × 40cm × 1m) with a grid was placed in each pen weekly, from the second week on. After four hours the excreta were collected from the container and stored in a freezer (-20°C). On day 36, the length of the *tarsometatarsus* of each bird was measured by flexing the leg and registering the distance between the medial condyle of the *fibula* and the *trochlea* for *metatarsus III*. The average was calculated per pen and the ratio of bodyweight to *tarsometatarsus* length was calculated as in Deeb and Lamont (Deeb and Lamont, 1998). Each week the consistency of the litter was observed by the same person.

Analysis

Both diets were analysed for dry matter (**DM**), ash, acid insoluble ash (**AIA**), crude fat (**EE**), crude fiber (**CF**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**) and crude protein (**CP**) (**Table 7.1.**). The DM and ash content were determined by drying the feed to a constant weight at 103°C and combustion at 550°C, respectively. The AIA content was determined using the procedure of Van Keulen and Young (Van Keulen and Young, 1977), as adapted by Atkinson *et al.* (Atkinson et al., 1984). The diethyl ether extract was analyzed with the Soxhlet method (ISO, 1973). Crude fiber was determined using the Association of Official Analytical methods (Method 962.09 and 985.29, 1995). To determine NDF and ADF, the methods of Van Soest *et al.* (Van Soest et al., 1991) were used. The Kjeldahl method (ISO 5983-1, 2005) was used to determine CP (6.25 x N). Excreta were freeze-dried at -50°C (Coolsafe, Labogene, Denmark) and homogenized. In the excreta, EE, CF, Ash and AIA were analysed as described above. The CP content (6.25 x N) (Kjeldahl method (ISO 5983-1,2005)) in the excreta of birds needed correction for uric acid (**UA**) as birds excrete faeces and urine together (Kalmar et al., 2007) (equation [[1]]). This was performed spectrophotometrically according to Terpstra and de Hart (Terpstra and de Hart, 1974). The external marker method with AIA as external marker was used to calculate apparent fecal digestibility as performed by Sales and Janssens (Sales and Janssens, 2003; Sales and Janssens, 2007) (equation [[2]], AFD_x, with x = EE, CF and CP). Based on the EE, CF and ash percentage of DM, ME was calculated according to Wiseman (Wiseman, 1987) (equation [[3]]).

Calculations

$$CP = (\text{total nitrogen} - \text{UA nitrogen}) \times 6.25 \qquad [[1]]$$

$$AFD_X(\%) = 100 - 100 \text{ x} \frac{\text{X\_excreta x AIA\_feed}}{\text{AIA\_excreta x X\_feed}} \qquad [[2]]$$

$$ME\ (MJ) = (3951 + 54.4EE - 88.7CF - 40.8Ash) \times 0.92 \times 0.004184 \qquad [[3]]$$

Statistical analysis

Statistical analyses were performed using RStudio (Version 0.98.507, RStudio Inc, 2009) and statistical significance was set at $P < 0.05$. For all analyses, pens were considered as the experimental unit and non-parametric statistics was performed for data analysis as normality of data could not be verified in the current trial set up. Both weekly bird weight (week 0 to 5) and digestibility of nutrients (week 2 to 5) were subjected to longitudinal non parametric analysis using the f2.ld.f1() function of the nparLD package with diet and breed as the whole-plot factors and time as the sub-plot factor. Decisions on significance were made based on the ANOVA type test statistics provided by the latter function (Brunner et al., 2002). To identify the effect of time, a pairwise comparison was done between the different time points by means of the mctp.rm() function of the nparcomp package. To estimate the effect of breed-specific growth rate and diet on average daily feed intake (**ADFI**), average daily gain (**ADG**), feed conversion ratio (**FCR**) and the *tarsometatarsus* length a linear permutation regression with 5000 replicates (lmp() of the lmPerm package) was performed. Each of the 16 pens was randomly assigned to one of the two diets. To differentiate the effect of diet and of breed-specific growth rate, the null hypothesis of no effect was used. The alternative hypothesis is that there is an effect of diet and breed-specific growth rate on the parameters (Higgins, 2004; Kabacoff, 2011). The maximum number of performed permutations was set at 5000 because of a total of 16! possible permutations for this data set. To exclude the possible error by only taking 5000 permutations the test was performed a thousand times with each time another set of 5000 random permutations. From these thousand permutation tests the maximum *P*-value was selected and represented together with the corresponding estimates of the linear regression which is presented as:

$$y = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij}$$

(with μ = intercept, α = breed-specific growth rate, β = diet, αβ = interaction growth rate × diet and ε = the random error term). The commercial diet was considered as the reference in this regression. Statistical significance was set at $P < 0.05$ and results were reported as mean ± standard deviation.

Results

Growth performance

A higher bodyweight was found with increasing time, increasing breed-specific growth rate and for the commercial diet (**Figure 7.1.**). For both diets, Cobb chickens achieved the highest bodyweight, each week. Sussex chickens always had the lowest bodyweight compared to the breeds with a higher breed-specific growth rate, but there was no significant effect resulting from diet on the bodyweight of Sussex chickens. Longitudinal analysis indicated a significant interaction of the factors breed, diet and time ($P < 0.001$) on the bodyweight.
The linear regression of ADG was determined by breed-specific growth rate ($P < 0.001$), diet ($P < 0.01$) and the interaction of both factors ($P < 0.01$) (**Table 7.2.**) (**Figure 7.2.**). The coefficient for the diet × breed-specific growth rate interaction was -0.39. This means that, compared to a commercial diet, the ADG of the chickens on a scavenger diet decreased with 0.39 g/ d when the growth rate increased with 1 g/ d.

Feed intake

The ADFI was significantly higher for the scavenger diet ($P < 0.001$) and with increasing breed-specific growth rate ($P < 0.001$). No significant interaction between breed-specific growth rate and diet on the ADFI was found ($P > 0.05$) (**Table 7.2.**) (**Figure 7.2.**).

Feed conversion

No significant interaction between breed-specific growth rate and diet on the FCR was found ($P = 1$). The scavenger diet significantly increased the FCR ($P < 0.001$) and a higher growth rate decreased the FCR ($P < 0.001$) (**Table 7.2.**) (**Figure 7.2.**).

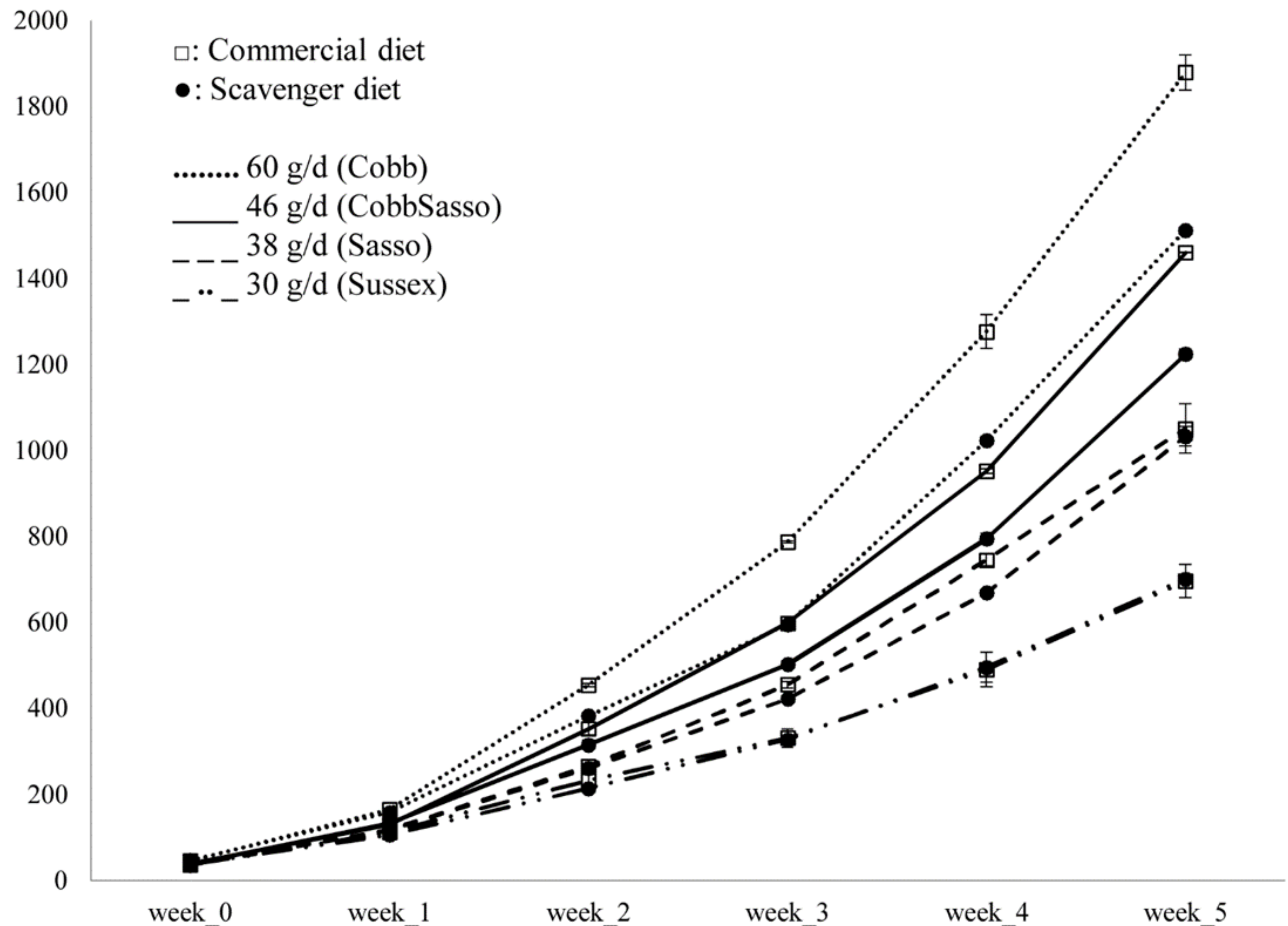

Figure 7.1. Bodyweight (± standard deviation) of four chicken breeds, each with its breed-specific growth rate, fed either a commercial or a scavenger diet.

Table 7.2. The linear regression between both factors (BS growth rate and diet) and ADG, ADFI, FCR, TMT and BW/ TMT

	Intercept	**BS growth rate**	**Diet**	**BS growth rate × Diet**	***R²***	***P***
ADG	30.4 ***	0.85 ***	-4.4 **	-0.39 **	0.97	***
ADFI	71.3***	1.3 ***	14.5***	-0.33	0.97	***
FCR	2.5 ***	-0.03 ***	0.81 ***	-0.01	0.88	***
TMT	8.3 ***	0.05 ***	-0.56 **	-0.03	0.82	***
BW/*TMT*	140.5 ***	3.1 ***	-8.7	-1.0	0.91	***

Coefficients of the linear regression of broiler chickens differing in breed-specific growth rate fed a commercial versus a scavenger diet correlated to the average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), length of tarsometatarsus (TMT) and the ratio between the bodyweight and the length of the tarsometatarsus (BW/TMT). The factors in the equation are breed-specific (BS) growth rate and diet. The commercial diet was considered as the reference. The BS growth rates for each of the breeds used in this experiment are: 60g/ d for Cobb, 46g/ d for CobbSasso, 38g/ d for Sasso and 30g/ d for Sussex. R^2 and the P-value of the linear regression models are given in the right colomns. Superscripts represent the P-value: * = <0.05, ** = <0.01, *** = <0.001.

Tarsometatarsus length

The length of the *tarsometatarsus* increased significantly with increasing breed-specific growth rate ($P < 0.001$) and when fed the commercial diet ($P < 0.01$). No interaction between the factors, diet and breed-specific growth rate, was detected. The results of the linear regression for the ratio of the bodyweight/ *tarsometatarsus* length were determined by the breed-specific growth rate only ($P < 0.001$) (**Table 7.2.**) (**Figure 7.2.**).

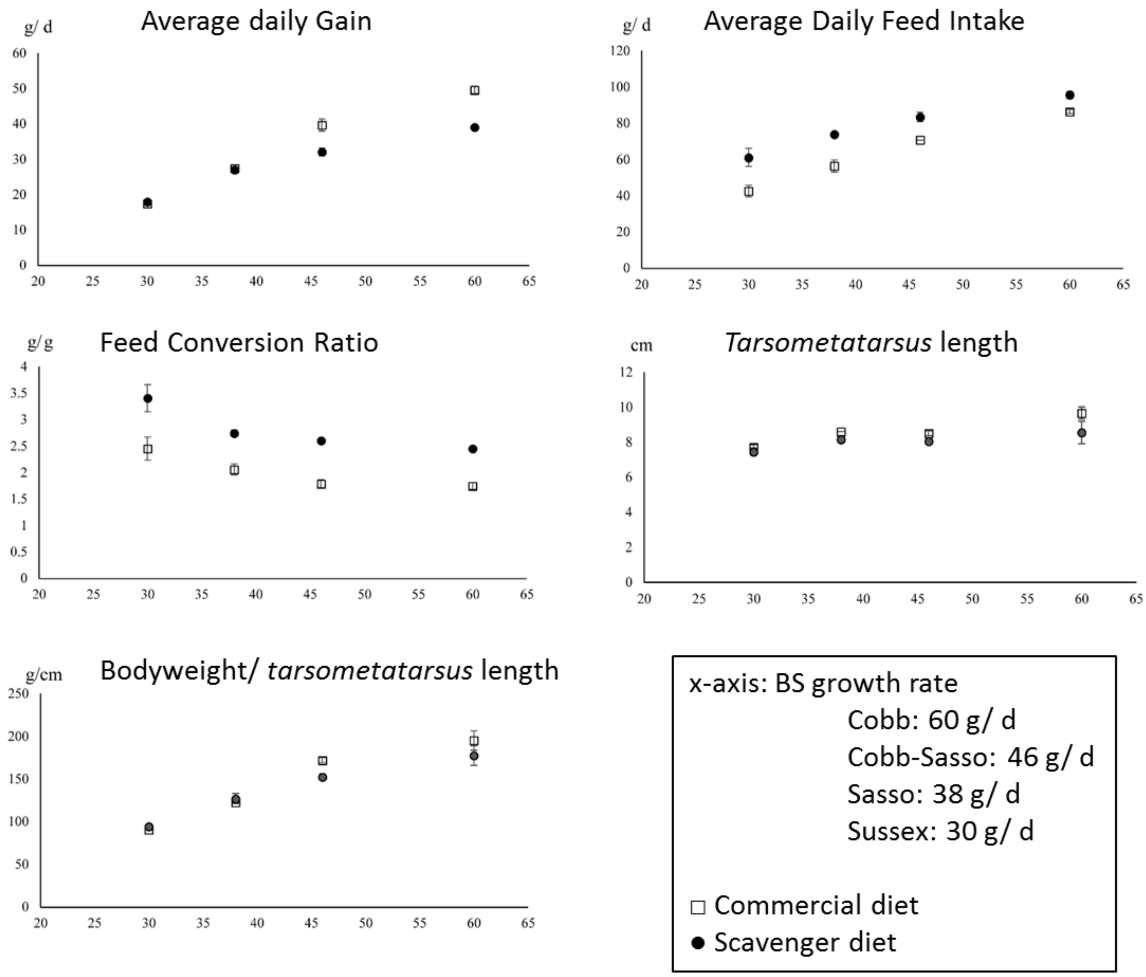

Figure 7.2.: The average daily gain, average daily feed intake, feed conversion ratio, length of the *tarsometatarsus* and bodyweight/ t*arsometatarsus* length (± standard deviation) of chickens fed the scavenger or the commercial diet in relation to their breed-specific (BS) growth rates.

Digestibility

The digestibility coefficients of EE and CP were lower on the scavenger diet compared with the commercial diet ($P < 0.001$ and $P = 0.004$ respectively). The EE digestibility of the commercial diet was 92% ± 3% and 82% ± 5% for the scavenger diet. The CP digestibility of the commercial diet was 51% ± 10% and 25% ± 22% for the scavenger diet. There was no significant difference ($P > 0.05$) found between the four breeds according to the digestibility of CF, EE and CP.

Litter

The litter was observed to be more humid and sticky with increasing breed-specific growth rate and when the birds were fed the scavenger diet (pers. Obs. JP).

Discussion

All breeds increased their ADFI when fed the scavenger diet. The two slowest growing breeds in this study, Sasso and Sussex, increased their feed intake by 40 and 30% respectively and therefore managed to achieve the same bodyweights as on the commercial diet. The bodyweight of Sussex was even slightly, but not significantly, higher when fed the scavenger diet. The fastest growing breeds, Cobb and CobbSasso, consistently achieved the highest bodyweights compared to the slower growing breeds. Yet, based on the FCR, they should have increased their feed intake by 45 and 40% respectively, in order to achieve the same bodyweights on the scavenger diet as on the commercial diet, which they were not capable of doing. This in contradiction to an experiment by Leeson *et al.* (Leeson et al., 1996a) where broilers of a commercial strain increased their feed intake sufficiently as the energy content of the feed decreased. These broilers did manage to maintain their bodyweight over an energy difference of 2.5 MJ. This contrast might be explained by the use of an “older” commercial broiler breed (which was not specified) or by the fact that the energy difference between the diets was lower than in our study, where it was 5.0 MJ/ kg. Olomu and Offiong (1980) found no significant effect on either feed intake or weight gain for starting broilers fed diets with an energy difference of 1.7 MJ. This in contrast to the study by Harms *et al.* (2000) where a significant effect ($P < 0.05$) of breed, diet and breed × diet interaction was found concerning both weight gain and feed intake. Equally so, Leeson *et al.* (1996b) noted an increase in feed intake as well as feed intake to bodyweight gain ratio of broilers when the energy and protein level in the feed were diluted. The chickens’ bodyweight was significantly affected on day 42 but not on day 49. No rating for the growth rates of the different chicken breeds was made in any of these studies.

The effects of the scavenger diet on bodyweight seen in our study are in line with Havenstein *et al.* (1994) and Grashorn (2006). In the first study, the effect of diet on the bodyweights of two chicken breeds, Ross 308 and Athens-Canadian Randombred, was measured. The diets were an industrial diet from 1957 and one from 2001, the latter one being higher in protein, fat and energy. For the Ross 308 breed a difference in bodyweight of 18.9 and 21.8% for females and males respectively was found and for the Athens-Canadian Randombred breed a difference of 5.4 to 7.8% for females and males respectively was found. The highest weights were always obtained for the 2001 industrial diet, but the differences with the 1957 industrial diet were smaller for the “older” breed. Grashorn (2006) found a difference in bodyweight of 8-14% for slow growing broilers, whereas for the fast growing broilers a difference of 25% between diets with a high and low nutrient concentration was seen. Both studies support the association between breed-specific growth rate and the difference in bodyweight when fed a less concentrated diet. None of those two studies monitored the digestibility of nutrients or the feed intake of the chickens.

The stable digestibility of EE and CP throughout time is in line with the results by Batal and Parsons (Batal and Parsons, 2002). In that study, an increasing digestibility of EE and CP was found up to the age of 14 and 10 days respectively. Later the digestibility coefficients reached a plateau. These results can be explained by the development of the small intestine, digestive enzymes and villus morphology between hatching and 6 to 14 days of age (Nitsan Z., Ben-Avraham G., Zoref Z., 1991; Sell J.L., Angel C.R., Piquer F.J., Mallarino E.G., 1991; Uni Z., Noy Y., 1995; Obst et al., 2014). In addition, the results of Jackson and Diamond (1996) and of Proudman *et al.* (1970) present equal (both 67%) apparent dry-matter digestive capacity for both jungle fowls and broilers for similar diets. This in contradiction with Krás *et al.* (2013) who found a significant, but “not biologically coherent” effect of age on the digestibility of DM, OM, CP, NDF, ADF and gross energy between the age of 10 and 41 days. The same study also showed a higher ADF-digestibility for the Label Rouge breed compared to the Cobb500 at the age of 31 and 41 days. It must, however, be considered that in the present study, the chicks on the scavenger diet only received 100% scavenger diet from day 11 on. Feeding the chicks 100% scavenger diet from day 0 might have given different result. Hence, to prevent health problems by very sticky droppings at such a young age, it was chosen to gradually introduce the scavenger diet.

In our study, the lower rate of fat and protein digestibility in the scavenger diet compared to the commercial diet could be explained by the high amount of NSP and the lack of added enzymes to break them down. The lack of enzymes enables the fibres to enclose the nutrients and keep them from enzyme break-down with their "cage-effect" (Knudsen et al., 1993; Jørgensen et al., 1996). The high CF content in the scavenger diet might also state the decrease in manure quality and increase in quantity, due to the water holding capacity of the fibres (Choct and Annison, 1992; Langhout and Schutte, 1996; Francesch M., 2004). The higher quantity of excreta in the pens where the birds were fed the scavenger diet might also be caused by the higher feed intake.

Though not significant, the effect of the scavenger diet on the *tarsometatarsus* length was higher with increasing breed-specific growth rate. This length was slightly, but significantly, higher for chickens with a higher breed-specific growth rate (P > 0.001) or when fed the commercial diet (P < 0.01). The ratio of bodyweight and a skeletal measurement, represented here as the length of the *tarsometatarsus*, can be considered as an indicator for the conformation of the body (Deeb and Lamont, 1998). The higher this ratio, the more weight per skeletal unit. A slightly, but not significantly, higher ratio of bodyweight/ *tarsometatarsus* length was found for the slow growing breeds on the scavenger diet compared to the commercial diet. This in contrast with Cobb and CobbSasso where this ratio was higher for the commercially fed chickens. The share of muscle, fat and digestive tract in the bodyweight was not determined.

An increasing FCR is generally considered an economic disadvantage but if the scavenger diet can be obtained at lower cost than the commercial diets, this perspective might change. For example, in a rural situation, where a scavenger diet can (partially) be found in the environment and is available at libitum, our results suggest that the slow growing chickens might achieve the same bodyweights as when they were fed a commercial diet. Still, factors such as disease, water availability and housing must be controlled (Awuni, 2002; Mwalusanya et al., 2002). In addition, chickens that are able to find (a share of) their feed in nature will minimize the competition between humans and livestock for feed/food resources. The results of this experiment also indicate that industrial broiler breeds will not be able to increase their feed intake sufficiently in order to grow as fast on a diet with lower energy and nutrient density (such as the scavenger diet in the present study) as they would do on a commercial diet. Extending the production cycle with ten days, however, is estimated to increase the price with 5% and an average consumer in the USA indicates to be willing to pay 35% extra (range between -29.6% and 244.3%) for more sustainable chicken meat (Moynagh, 2000; Van Loo et al., 2011). When using the FCR data from this study, a Cobb chicken would nearly need twice the amount of scavenger feed compared to the commercial feed to reach 2.2 kg of slaughter weight. This indicates that the scavenger diet must be cheaper than half of the commercial diets' price in order to make the same profit. This calculation is only an estimate as the number of production cycles a year, transportation costs and others are not taken into account.

Conclusion

The advantage of selecting fast growing broiler chickens on concentrated diets decreases rapidly when less concentrated diets are given. This urges us to reconsider the current selection criteria when considering increasing the amounts of by-products in poultry feed as driven by the feed-food competition. For slow growing chicken breeds – such as the ones used by smallholders in developing countries – the added value of using a concentrated diet versus a typical scavenger diet is low. These differences in growth performance are caused more commonly by differences in feed intake than by differences in digestive capacity.

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Chapter 8

General discussion

Take home messages from the studies

1. Human interference induces a shift from a fibrous, low-protein and low-energy chicken diet to a low-fibre, high-protein and energy-rich chicken diet (**Chapter 3**).
2. Many different ingredients were found to be ingested by free-roaming, rural chickens and insects were found in nearly each crop (**Chapter 3**).
3. Rural chickens in Limpopo, South-Africa are managed with a minimum of time, money and effort. Both scavenged and supplemented feed is needed for the chickens to survive but starvation is rarely a cause of death (**Chapter 4**).
4. Cecal drop is a good representative of the cecal microflora regarding bacterial community, richness and diversity and fecal drop is not (**Chapter 5a**).
5. The scavenger diet showed an increase in bacterial diversity and a change in the community composition compared to the commercial diet (**Chapter 5a**).
6. Ten bacterial genera were found to be indicative for the scavenger diet and two for the commercial diet (**Chapter 5b**).
7. Chitin biases the results for crude protein analysis. However, the level of nitrogen derived from chitin in insects is quite low compared to the overall nitrogen content (**Chapter 6**).
8. The superiority of fast growing breeds compared to slow growing breeds decreases when fed a scavenger diet compared to a concentrated diet (**Chapter 7**).

General discussion

Decreasing the share of food in the feed of chickens, is not a goal on itself. Decreasing the feed-food competition is about increasing the use of human-inedible sources and reducing the spillage of ingredients that can still be used in feed. This in order to increase the food security for a growing human population all around the world. In this dissertation, rural chickens were observed as a model for a minimal feed-food competition (**Chapter 3**). Observing the scavenging diet of free-ranging chickens taught that chickens ingest a huge variety of ingredients such as seeds, insects and green forage, and that they do not avoid fibrous ingredients, while fibre is generally considered unimportant in commercial poultry diets. Two points of interest were subsequently identified.

The first one is the fibre content. As fibre fermentation is done by bacteria in the ceca, studies on the cecal microbiome of chickens will be important in the future (**Chapter 5a**). The second point concerns insects. They are believed to be one of the most promising alternative ingredients of the future. Nevertheless, in order to analyse digestibility and nutritional composition of insects or samples that contain insects, specific methods will be necessary (**Chapter 6**).

As the chickens observed in **Chapter 3** are all slow growing, rural chickens, questions rise how high productive, fast growing chickens will perform on low concentrated diets. **Chapter 7** showed a linear relation between the growth rate and the effect of a scavenger diet on weight gain. Sussex, a slow growing breed, achieved the same growth rate on both diets. Although Cobb, the fastest growing breed, still grew fastest of all breeds and regardless the diet, its superiority decreased. This indicates that the best performing breeds in commercial situation should not necessarily be the breeds of choice when the diet changes.

As no effect of diet could be shown on the growth rate of slow growing chickens (**Chapter 7**), other points of focus should be identified in the attempt to increase the profit that rural chicken owners make of keeping chickens. Rural chicken owners in Limpopo, South-Africa, were questioned about the management of their chickens and the ingredients in their crops were identified (**Chapter 4**). Disease prevention and protection of the chickens against predators are important points of focus when attempting to increase the profit for rural chicken owners in this area.

On the one hand, rural chicken owners in developing countries might feed their chickens with local ingredients in order to decrease the effect of currency devaluation and import costs (Hutagalung, 2000; FAO, 2002). They can choose to use local breeds, even though they grow slowly and have a low productivity, because their meat is more appreciated by the consumers and they are able to take care of themselves and their offspring in extensive situations (Pym, 2008; Alders and Pym, 2009). Farmers who grow broilers in an intensive management system, on the other hand, maximize their profits by using breeds that grow extremely fast on diets that contain high levels of highly digestible protein and energy (Gill et al., 2010; Snels, 2012; Tscharntke et al., 2012), although, the prices of these diets are high and these chicken breeds often cope with health problems related to their high productivity (Donohue and Cunningham, 2009; Kalmar et al., 2013). Farmers in general, however, will only change their management if, for example, the feed costs or the health of their chickens, increase the profit. Furthermore, as the feed prices increase over the years, a tipping point might arrive where a lower production is justified by the lower costs of feed ingredients.

When attempting to decrease the feed-food competition, many factors are involved. This PhD thesis highlights some of them, however, we fully acknowledge that this list is not complete and, therefore, end with suggestions for further research.

8.1. Methods to decrease the feed-food competition in the chicken industry

Lower the fibre content in fibre-rich ingredients

Obtaining complete guidelines regarding fibre, and especially the different fibre fractions, in the feed of chickens is difficult (NRC, 1994; DeVries, 2003; Hall, 2003). It is even more complicated as the optimal content and composition of fibre fractions in the diet will change with breed, age, housing and health of the chickens (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2010; Mateos et al., 2012). Yet, high fibre contents will be one of the biggest challenges when incorporating alternative ingredients in the chickens' diets. Many of the suggested alternative ingredients (**Appendix 1**) and nearly all diets of free roaming chickens, contain high levels of fibre compared to the commercial diets (up to 10% *versus* 4%) (**Chapter 3**). None of the studies considered in Chapter 3 properly reported the content of the different fibre fractions in the studied diets.

Although the contents of different fibre fractions in the scavenger diet and the capabilities of rural chickens to deal with them are still unclear, reducing the fibre content of fibrous ingredients will benefit the performance of the chickens (Kalmendal et al., 2011). The energy that chickens can obtain from non-starch polysaccharides (NSP) rich ingredients is negligible (Jørgensen et al., 1996). In addition, NSP can cause a “cage-effect” and, therefore, prohibit the accessibility by enzymes to other nutrients (Bedford, 2002). Decreasing the fibre content in the alternative ingredients can be done with a low risk of decreasing the fibre to a suboptimal level as 1) green forages, leaves, seeds and/ or other fibrous ingredients, can easily be provided *ad libitum* and 2) some alternative ingredients (**Appendix 1**) and scavenger diets (Tadele, 1996) show very high levels of crude fibre.

Different methods to decrease the fibre in the chickens’ diet have been applied before. Enzymes such as xylanase are a standard add-on in the commercial diets based on wheat (Bedford and Schulze, 1998; Hetland and Svihus, 2001; Choct, 2006; Cowieson et al., 2006). They increase the accessibility of the endogenous enzymes to the starch, therefore reducing the cage-effect caused by the fibres (Bedford, 2002).

The cecal microbiota are responsible for the breakdown of fibres in the diet (Dunkley et al., 2007) and are linked with the feed conversion of the chickens (Stanley et al., 2012). However, no differences in cecal microflora between breeds have been found in our study (**Chapter 5a**). Studying the cecal microflora of rural chickens, not only exposed to a scavenger diet since the day they hatched, but also descending from generations of free roaming chickens, therefore possibly adding a heritable effect (Amerah et al., 2009; Anderson et al., 2012), might provide other results. Based on evolutionary fitness (Darwin, 1859), it can be expected that the cecal microflora of rural chickens substantially differs from the one of commercial breeds and promotes better fibre fermentation because 1) the cecal microflora is responsible for the fibre degradation (Mathlouthi et al., 2002; Dunkley et al., 2007), 2) the scavenger diet is high in fibre

(**Chapter 3**), and 3) the microflora of the chicks is determined by the environment and the gut microflora of the mother (Amit-Romach et al., 2004). In addition, the scavenger diet increases the diversity and richness of the cecal microflora (**Chapter 5a**), though, the effect of this change on digestive capacity or health could not be ascertained (**Chapter 5b**). According to some authors, the capacity to digest fibre increases with the age of the chicken (Carré et al., 1995) but this is contradicted by others (Kras et al., 2013). Inoculating commercial chicks with the microbiota of adult, rural chickens, might be one of the strategies to increase the use of fibre in the diet. Nurmi and Rantala (1973) showed that by inoculating chicks with the cecal content of healthy adult chickens, they are protected against *Salmonella* infection. So far, no studies have monitored the effect of transplanting the cecal microflora on the breakdown of fibres.

When comparing the microbiota of children in Burkina Faso to European children, big differences were found because their diet substantially differed (De Filippo et al., 2010). The diet of the children in Burkina Faso was higher in fibre and lower in fat, animal protein, calories, sugar and starch compared to the European diet. The evolutionary similarity with the diets of industrial and rural, indigenous chickens is clear (**Chapter 3**). The microbiome of children in Burkina Faso was richer and more diverse, which was also seen when comparing chickens on a commercial and on a scavenger diet (**Chapter 5a**). For the children of Burkina Faso, more SCFA were produced, which are known to benefit the gut health and to protect against colonization of pathogenic species (De Filippo et al., 2010). For chickens too, SCFA are proven to stimulate the chickens' health by providing energy to enterocytes and inhibiting the colonization of pathogens by decreasing the pH (Patterson and Burkholder, 2003). Another human study linked lower fibre in the diet to "Western" diseases such as ischemic heart problems, high serum cholesterol levels and gallbladder diseases (Burkitt et al., 1974). For these diseases too, there is a similarity with industrial chickens as they suffer from ascites, heart conditions and fatty liver (Kalmar et al., 2013; Rozenboim et al., 2016). A study in Nature discovered that mice, fed a low fibre diet, not only lost a share of their microbiota richness, this loss was also irreversible when increasing the fibre in the diet again. Furthermore, this loss was passed on to their offspring (Sonnenburg et al., 2016). These results alarmed the authors as similarities with the Western human situation are obvious. For chickens too, it can be expected that indigenous, free-ranging chickens might be important in the future as microbiota donors for the industrial chickens. Not only in terms of fibre digestibility, but also to increase their overall health.

Another way of decreasing the level of fibre is to include an extra step in the feed processing. Insects convert grass, kitchen waste, leaves and even manure, to protein and fat. Rather than giving this organic material directly to the chickens, it would be interesting to let insects decompose it first and, afterwards, feed the insects to the chickens (Awoniyi et al., 2003; Diener et al., 2009; Makkar et al., 2014; Maurer et al., 2015). In the same idea this “processing” of waste can also be done by bacteria. Positive effects of adding bacteria protein meal to the diet of broilers have been demonstrated (Øverland et al., 2010 a-b).

Reducing the fibre content in fibrous feed ingredients will generally benefit the performance of the chickens, however, one should not forget the benefits of fibre on the welfare and health of the chickens. It improves the function of the gizzard (Jiménez-Moreno et al., 2010), increases the digestibility of fat and protein (Alzueta et al., 2010), enriches the cecal microflora (**Chapter 5a-b**) (Mathlouthi et al., 2002; Wang et al., 2005) decreases hunger (Jones et al., 2010) and increases immunity (Schley and Field, 2002; Guo et al., 2003). Still, these effects are specific for the study setup, for a particular source of fibre, inclusion level, age of the chickens, … (Jørgensen et al., 1996; Jiménez-Moreno et al., 2010; Mateos et al., 2012). These effects, and many more, could increase the welfare of chickens in the industry and are important to rural chickens, where drugs are not standardly available and optimal use of the nutrients in the diets is essential (Goodger et al., 2002).

Which breeds to use?

Slow growing breeds show more resilience to a challenging diet compared to fast growing breeds (**Chapter 7**). This is in accordance to previous studies (Havenstein et al., 1994; Grashorn, 2006), where “older” or “slower growing” breeds were less affected by an energy low diet compared to the industrial breeds. However, even on the scavenger diet, the fast growing breeds still reach the highest body weights (**Chapter 7**). These results can be interpreted in two ways: one can conclude that rural chicken owners will benefit from exchanging their slow growing breeds for fast growing chicken breeds. Or, one can conclude that owners of slow growing chickens can reduce their feed costs by exchanging the grains and commercial diets for locally available substrates. Feed costs in the chicken industry occupied 68.7% of the production costs in 2008 and feed prices are shown to increase over the years (**Figure 8.1.**) (Hinrichs and Steinfeld, 2007; Donohue and Cunningham, 2009).

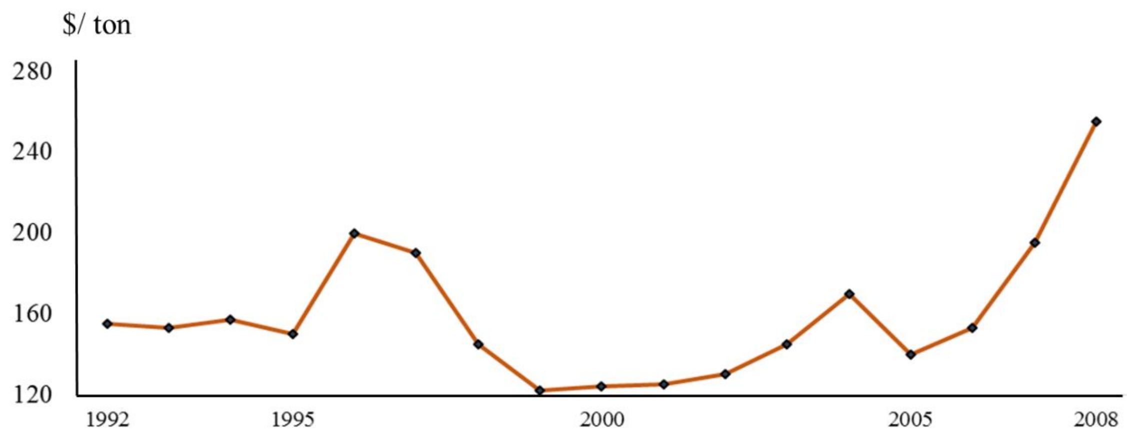

Figure 8.1. Feed cost per ton for the US broiler industry (Donohue and Cunningham, 2009).

Slow growing indigenous breeds cannot be replaced by fast growing industrial chickens without consequences. Features, important to survive in rural circumstances, might have been lost during “unconscious” selection (Darwin, 1859). For example, a negative relation was found between growth rate of chicken breeds and their immunity (Qureshi and Havenstein, 1994; Van Der Most et al., 2011). Additionally, when White Leghorns are compared to red junglefowl, the foraging strategy of the Leghorn was less active, the Leghorn was less responsive to fear tests with a hawk model (Schütz et al., 2001), and red junglefowl were able to process more information during foraging and forage more intensively for their feed (Lindqvist et al., 2002). Although not confirmed by the results in **Chapter 7** of this thesis, a better fibre diet digestibility was found for the slow growing Label Rouge, compared to fast growing Cobbs (Kras et al., 2013). All these features are important for chickens in rural situations.

In addition, the meat and eggs of indigenous chickens are preferred by the local people, who are willing to pay a surplus for them (Guèye et al., 1997). Indigenous chickens play a cultural role in ceremonies or as gifts and they provide an immediately available source of money in case of financial problems. **Chapter 4** showed that the main reasons why rural people in Limpopo, South-Africa, kept chickens were: meat for own consumption, reduction of kitchen waste and to have some backup money. Selling and consumption of eggs were indicated as secondary reasons only. Most of the chickens kept in the complete free-range system are in hands of women, providing an income for them and therefore contributing to gender equality (Ekue et al.; Guèye, 2003; Guèye, 2005). This is in contrast to more intensive systems which are often under the control of business men. In addition, slow growing breeds can be managed with a low input, making it possible for the poorest to provide an animal protein source for their families (Guèye, 1998; Sonaiya et al., 1999; Henning et al., 2007). In developing countries, especially for young children, both the physical and mental development are strongly correlated with the level of animal products consumed. These animal products provide extra protein, but their biggest value lies with the provision of extra micro-nutrients (mainly vitamin A, vitamin B_{12}, riboflavin, Fe, Zn and Ca) (Calloway et al., 1988; Allen et al., 1992; Murphy and Allen, 1996). This illustrates the importance of an affordable animal component in the diet of people in developing countries.

When keeping slow growing, rural chickens on a scavenger diet, many points will require attention. As shown in **Chapter 7**, the feed intake of the chickens increases when they shift to a diet that is lower in energy and protein. Therefore, enough quantity of feed must be assured in order to reach the maximal productions for a particular diet-breed combination. None of the reviewed studies on the chickens' natural feed, and our study neither (**Chapter 3-4**), included information about the absolute amount of feed ingested. The relative composition of the diet was presented, but this included only percentages and no daily volumes. It will technically be a challenge to obtain these data in a situation where chickens roam around freely (Yo et al., 1997), though, absolute quantities might be more determinative to the chickens' performance compared to the nutritional composition of the diet (**Chapter 7**). Of course, to optimize performance in rural environments, other basic needs such as disease control and predator control should also be taken care of (Alexander, 2001; Awuni, 2002)(**Chapter 4**).

Progresses in the fields of disease control, housing, management and others all contributed to the industrial production results of today. When increasing the use of alternative ingredients, these aspects might have to change too. It would be interesting to reconsider the industrial breeds and select chickens towards performance on low energetic diets. In that case, some management factors might have to change too. It might, for example, be more economical to rely on the feed flexibility of the chickens seen in **Chapter 3 and 4**. Anticipation to available ingredients at the moment will be quicker, as no time and money will need to be spent on preparing the “perfectly balanced” feed. Chickens can combine available protein sources to maximize performance (Shariatmadari and Forbes, 1993; Gous and Swatsom, 2000), in addition, their diet choice might be influenced by factors such as sex, age and health depending on their specific need (Savory et al., 1978; Arshad et al., 2000; Minh et al., 2006). Not only will chickens have the opportunity to compose their nutrient profile as they desire it will also increase their welfare as picking, scratching and selecting their feed is close to their natural behavior (Costa et al., 2012). Fanatico et al. (2016) showed an improved FCR for organic chickens that were choice fed compared to a more expensive formulated diet. In addition, chickens can also limit their protein intake if ingredients, (too) rich in protein, are offered (Kim, 2014).

Insects, alternative ingredient of choice?

As insects are found in the crop of nearly all chickens that were sampled (**Chapter 4**) and as they were recited in each study that focusses on the diet of rural chickens (**Chapter 3**), they cannot be overlooked as alternative ingredients in the chicken’s diet. They do not only provide animal protein and fat, they can also reduce waste products. For example, black soldier flies can grow on fish offal and cow manure (St-Hilaire et al., 2007) and an inclusion of 12% house fly larvae meal, grown on municipal organic waste, did not affect final weight or feed conversion, compared to a soybean meal diet (Ocio et al., 1979). Furthermore has it been proven that insects reduce the number of bacteria in manure (Erickson et al., 2004).

Still, before the European Union will allow insects in the feed of livestock, much more research will have to be completed. In general, the European legislation about insects as feed and food is confusing as it is not clearly stated in the law whether insects are “novel food” and whether they are considered animals when they are included in the diet (Belluco et al., 2013; van der Spiegel et al., 2013). The EFSA wants to be sure that no health risks are involved for the consumers, consuming insects or livestock fed with insects. Therefore all scenarios of allergies, disease transmission and toxic components have to be studied thoroughly (Adamolekun et al., 1997; Linares et al., 2008; Klunder et al., 2012). Information about specific rearing methods (Sheppard et al., 2002; Tomberlin et al., 2009), processing and storage (Klunder et al., 2012), preservation of the nutrients (Kinyuru et al., 2010) and many more, is needed before insect breeding for industrial purposes can be profitable. In addition, when including insects in the feed, nutrient composition, availability and digestion will have to be tested too (Banjo et al., 2006; Finke, 2007; Oonincx and Dierenfeld, 2012; Pieterse and Pretorius, 2013).

Getting a correct view on the different nitrogen fractions in chitin-containing diets is not easy (**Chapter 6**). Chitin biases the results of the crude protein analysis by the Kjeldahl method because it contains nitrogen. However, the level of chitin in insects is not found to be extremely high (**Chapter 6**). Therefore, it can be concluded, in accordance with Finke (2002), that the protein content, evaluated by the Kjeldahl method, does not significantly deviate from the actual protein content. In 2007, Finke even reported lower levels of chitin (Finke, 2007), but this is probably due to a mistaken citation. In an alternative diet, where insects only represent one of the ingredients, the nitrogen derived from chitin will represent an even be smaller share of the total nitrogen content. Yet, other sources of nitrogen, such as DNA, vitamins and amides, can also be present. The results of **Chapter 6** indicate that this share of nitrogen is bigger than the one derived from chitin.

Even when the nitrogen in chitin does not substantially causes an overestimation of the protein content in insects, it might still decrease the availability of the protein present. As chitin is stored in the cuticle, which has the function of an exoskeleton, the question rises whether other nutrients are still accessible for the enzymes in the gut (Hossain and Blair, 2007). This “cage-effect” has been described for fibres in the cellwalls of grains before, where it is often bypassed by adding enzymes to the diet. Puncturing the exoskeleton improved the bioavailability of the nutrients in crickets and mealworms (Prinz et al., 2003). However, in free roaming chickens, insects in the crop are rarely found in pieces (**Figure 8.2.**). Chickens appear to fully swallow their feed, without picking it to pieces first. In order to get to the nutrients captured by the exoskeleton, they thus have to rely on enzymes to digest the chitin and/ or have to mechanically crush the insects in the gizzard.

Figure 8.2. Intact invertebrates found in the crop of scavenging chickens in Limpopo, South-Africa.

Endogenous chitinase, the enzyme that digests chitin, is found in the proventriculus, gizzard and liver of chickens (Han et al., 1997; Suzuki et al., 2002; Koh and Iwamae, 2013). Chitinase producing bacteria in the microflora have been reported too (Whitaker et al., 2004), however, to our knowledge, never in chickens. A chitin digestibility of 92% was found for laying hens (Hirano et al., 1990), though the experimental design of this study is not completely clear. Much lower chitin digestibility was found for a study on broilers where excreta were collected for three subsequent days. A maximum of 24% was found for a 1.3% chitin diet, and the chitin digestibility decreased with increasing chitin inclusion (Khempaka et al., 2006). Hossain and Blair (2007) described a digestibility between 45% and 50% for chitin inclusion between 2.5% and 7.5%.

Both positive and negative effects of chitin on the performance of chickens have been reported (Ramachandran Nair et al., 1987; Okoye et al., 2005; Khempaka et al., 2006). Chitin has been suggested to not provide energy (Weiser et al., 1997) but to benefit the performance of chickens by stimulating immunity (Huang et al., 2007), increasing feed intake (Ramachandran Nair et al., 1987) or by acting as a prebiotic by increasing *Lactobacillus* and decreasing *E. coli* in the cecum (Li et al., 2007). When feeding chickens a scavenger diet, containing mealworms, *Lactobacillus* was found as an indicator genus for the scavenger diet (**Chapter 5b**). Some *Lactobacilli* spp. are proven to have probiotic effects in the cecum of chicken as they produce lactate and propionate and stimulate butyrate-producing bacteria (Meimandipour et al., 2009). Although *E. coli* is present in the intestines of healthy chickens, pathogenic strains can cause big losses. In addition, there exists a high prevalence of antibiotic resistance among *E. coli*. Therefore, extra options, such as chitin, to keep *E. coli* low, are valuable (Dho-moulin and Fairbrother, 1999).

8.2. Advantages for farmers and chickens

The increasing demand for grains in the fuel, food and feed industry has nearly doubled the live production costs for broilers in the USA (from $0.25 to $0.45 between 2006 and 2008) (Donohue and Cunningham, 2009) (**Table 8.1.**). When asking rural chicken owners in Limpopo, South-Africa, about their main reasons for not keeping more chickens, 53.6% agreed that the feed expenses are too high (**Chapter 4**). Offering alternative feed ingredients, less wanted in the human diet or in the fuel industry, will allow those farmers to decrease their feed costs, even if only partial.

Table 8.1.: Components of live production costs of broilers in the USA as a percentage of total production (Donohue and Cunningham, 2009).

	2001	2007	August 2008
Chick cost	16.2	13.4	10.9
Grower cost	19.9	17.0	11.8
Feed ingredient	51.8	59.1	68.7
Mill and delivery	4.3	4.3	3.4
Vaccine & medicine	0.3	0.2	0.2
Live haul	5.2	4.4	3.5
Others	2.3	1.7	1.5

Hutagalung (2000) studied the poultry industry in Indonesia and concluded that low-protein diets, supplemented with essential amino acids, provided a significant profit margin, although production performance was lower compared to conventional methods. Equally so, compared to conventional methods, these farmers were less dependent on global price fluctuations, currency devaluations and import costs, the latter often being too high for the poorest countries (FAO, 2002).

People in developed countries are willing to pay a surplus for organic poultry meat, based on their concern for their health, animal welfare and environment (Oberholtzer et al., 2006). It is estimated that breeding broilers that are ready for slaughter at 50 days instead of 40 days will increase the overall production price with 5% (Moynagh, 2000), opening possibilities to include slower growing breeds in the industry as the average consumer in the US is willing to pay 35% extra (range between -29.6% and 244.3%) (Van Loo et al., 2011). The surplus that consumers are willing to pay for organic food increased over the years together with the demand for organic poultry meat (Oberholtzer et al., 2006).

Alternative diets, often high in fibres, might benefit the welfare of the chickens (reviewed by Hetland et al., 2004) as fibre is shown to reduce hunger and therefore reduce cannibalism (Hocking et al., 2004), stimulates the development of the digestive organs, both mechanically and by the production of SCFA (Eeckhaut et al., 2008; Jiménez-Moreno et al., 2010) and boosts the immune system (Schley and Field, 2002). When offered a choice, chickens, raised on grains, equally consume insects, fruits (*Actinidia arguta*) and grains, although some time for adaptation was needed (HoGent, 2015). Reverting to breeds that are less selected for high performance on energy-rich diets, can solve some health problems that are regularly seen in the industry. Selection caused, for example, a decrease of 20% to 33% of the lung volume per bodyweight unit and makes these chickens, therefore, more vulnerable to stress factors (Vidyadaran et al., 1990). Van der Most *et al.* (2011) found that selection for growth compromised immune function of chickens, meaning that fast growing breeds depend more on antibiotics and therefore contribute to the overall problem of antibiotic resistance for both animal and human (reviewed by Silbergeld et al., 2008). In addition, drug residues in meat and eggs are a huge concern (Nisha, 2008). A survey in Iraq found residues in 52% of the meat pieces tested (Shareef et al., 2009). Furthermore, the use of antibiotics can also be reduced as fibres will improve the gut health through production of butyrate and other SCFA (Eeckhaut et al., 2008).

8.3. The opposition

Reducing the feed-food competition by reducing the share of human-edible feed might sound evident, but many authors conclude differently. It has been suggested that decreasing the share of cereal grains in the feed will decrease the cereal prices and will stimulate farmers to shift to higher profit crops, bypassing the idea of increasing the availability of cereal grains to the people (FAO, 2011). Westhoek *et al.* (2011) calculated that when livestock would only be fed grasses and by-products, less than half of the livestock could be maintained and Keyzer *et al.* (2005) concluded that animal production on residue-based feed alone will only be possible when the demand for meat is low. The FAO (2011) concluded "there is no technical or economical alternative to intensive production for providing the bulk of the livestock feed supply in the future". Bradfort *et al.* (1999) stated that research into ways of supplying more cereal grains will be needed anyway.

Considering these opinions does not conclude that alternative ingredients will not be useful. As chickens represent such a high share of the global livestock (**Table 8.2.**) and as they are the livestock species that consumes most human-edible feed (Wilkinson, 2011), even small improvements will substantially contribute to sustainable feeding. When only a share of cereal grains can be replaced by alternatives in the chickens' diet, a substantial amount of cereal grains will become available to be used as food. Possibly, when the demand for feed-grains decreases, the prices will drop and grain farmers might switch to higher priced crops. This is, however, not necessarily a disadvantage as the supply of those high-priced crops will increase and therefore those prices might drop, hence promoting the accessibility of human food sources after all. A decreasing demand for cereal grains in feed might also be buffered by a concomitant rise of cereal demand in the bio-fuel industry.

Table 8.2. Total number of livestock species, cats and dogs, and humans in the world (Leenstra and Vellinga, 2011; FAOSTAT, 2015)

Species	Total number in the world
Chickens	21.3 billion
Humans	7.3 billion
Sheep & goats	2.2 billion
Cattle	1.5 billion
Pigs	986.6 million
Cats & dogs	438 million
Horses	58.9 million

Many of the suggested alternative ingredients are higher in fibre, have a lower protein content or a less suitable amino acid profile compared to cereal grains and soybean meal (**Appendix 1**). Others, however, strongly match the chickens' requirements. Makkar *et al.* (2014) reviewed the possibilities of different insect species in feed by comparing them to fishmeal and soybean meal and concluded that they are of perfect nutritional quality to be included. Furthermore, increasing more fibrous ingredients in the diet should not plainly be considered negative. Benefits on health, digestibility and animal welfare by increasing the fibre content in feeds have been discussed higher and hypotheses of increasing the fibre digestibility by transplanting cecal microbiota or crossbreeding with rural breeds have been stated. In addition, protein and vitamin guidelines are often overestimating the chickens' needs. This does not only unnecessarily decrease the applicability of alternative ingredients, it also increases the burden on the environment as more nitrogen will be released.

Other solutions to decrease the feed-food competition than only decreasing human-edible ingredients in livestock feed should be considered too. One third up to half of the food after harvesting is reported to be wasted (Lundqvist et al., 2008; Gustavsson et al., 2011). Developing countries lose over 40% of food post-harvest or during processing because of storage and transport conditions. Industrialized countries have lower producer losses, but at the retail or consumer level more than 40% of food is wasted (Gustavsson et al., 2011). Improving infrastructure, raising awareness among the consumers and increasing the use of rejected food and leftovers, might help to reduce the waste. Boonen (2015) states that agriculture should focus on the needs but acknowledges that "wealth encourages creations of desired demands". Meat consumption in the Western world, for example, has far exceeded nutritional needs (Westhoek et al., 2011). As much as decreasing meat consumption in these countries would be beneficial, this cannot be extended to the whole world as animal protein, meat included, plays an important role in a balanced diet (Murphy and Allen, 1996). Even in developed countries, vegetarianism is not the solution either. Although a balanced diet without meat can be composed in developed countries, an increased amount of female animals would be needed to produce enough eggs and milk. Today, two thirds of the beef in Europe comes from dairy cows (Topliff et al., 2009). The biggest influence, however, lies with the consumer. Paying a fair price for local, healthy and sustainable products will benefit all parties.

8.4. Shortcomings, research limitations and future research

Based on the studies described in Chapters 3-7, some suggestions for further research can be made. Information on the diet choice of chickens, without human interaction, was very difficult to obtain (**Chapter 3**). Only one study could be found (Savory et al., 1978) in which chickens were not supplemented. Studying chickens, living away from crop fields or other human provision, would give new insights. Not only the ingredients they ingest, but also the absolute volume of feed they consume per day and the nutritional composition, with extra attention for the different fibre fractions, would be very interesting.

Composing a diet for the trial described in **Chapter 7**, as a representative of a scavenger diet, was a challenging mission. Fibre-rich ingredients and insects were included as they were found in the scavenger diets described in Chapter 3. However, the protein content of the scavenger diet was still quite high (18.7%) and the chicks on the scavenger diet got the chance to gradually adapt to the scavenger feed. In addition, chickens that roam around seldom have their feed freely available, where in this study the feed was available ad libitum.

No difference in digestive capacity or cecal microflora between four breeds could be found (**Chapter 5a and 7**). However, an extra trial, with more extreme breeds (indigenous chickens for example), might show differences in digestive capacity and/ or cecal microflora. When chickens are found, able to digest fibre to a bigger extent, one can proceed with in vitro studies and inoculation trials. When performing an in vitro study, the digestibility of pure fibre samples, including chitin, can be analysed.

Selecting chickens to perform on alternative ingredients would be a long term project. But once a chicken breed is developed, able to optimize its production fed on alternative ingredients, both developing and developed countries might benefit.

The value of sensitizing consumers about the whereabouts of their food, both in developing and developed countries, should not be forgotten. Reducing waste, using local products and taking care of the environment are things that all of us can apply on a personal level. All these matters, reducing the feed-food competition included, will contribute to a sustainable world for the future.

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Appendix

Appendix 1: List of reported alternative ingredients for broilers and/ or laying hens

Bacterial protein meal	Improved feed conversion in broilers and did not affect weight gain compared to soybean control diet (Øverland, Schøyen, et al., 2010).
Broken rice	Crude protein content of 9.8%, ADF and NDF low. But lysine nearly eight times lower compared to soybean meal (Mutayoba et al., 2011).
Canola meal	Contains anti-nutritive factors such as glucosinolates, sinapine (can induce fishy taint in the eggs), phytic acid and tannins and dietary fibre is high. Amino acid composition complements with soybean meal (Khajali and Slominski, 2012). Feed conversion in broilers (4 - 41 d.) improved with a diet of 400 g/ kg Pinjarra canola meal (replacing 148g /kg of soybean meal and 424 g/ kg of wheat in the control diet) although weight gain decreased (Perez-Maldonado et al., 2002).
Chick peas	Crude protein content of 166 g/ kg fresh chick peas (of which 10.8 g/ kg lysine) and tannins present (Farrell, Perez-Maldonado, et al., 1999).
Cottonseed meal	Feed conversion in broilers (4-25 d.) improved with a diet of 400 g/ kg cottonseed meal (replacing 250g /kg of soybean meal and decreasing about 250 g/ kg of sorghum in the control diet) although weight gain decreased. For laying hens, an inclusion of 200 g/ kg cottonseed meal caused a 10% decrease in egg productivity (18 – 57 weeks of age) (Perez-Maldonado, 2003).
Curcubita seed meal	Crude protein content of 34.10% with lysine of 1.56% DM but presence of trypsin inhibitor (Mutayoba et al., 2011). Inclusion of 66 g/ kg *Cucurbita* seed meal (replacing soybean meal and vegetable oil) improved feed conversion and increased live weight. Abdominal fat and harmful lipids in the serum were reduced by inclusion of 100 g/ kg. There was no effect on meat sensory quality (Aguilar et al., 2011).
Distillers dried grains with solubles (DDGS)	Body weight and feed conversion were not affected after inclusion of 9% DDGS in the diet of broilers (1- 4 wks) compared to a control diet that was higher in corn and soybean meal (Shalash et al., 2009). Inclusions up to 24%

	for corn DDGS (Shim et al., 2011) and 20% for wheat DDGS (Thacker and Widyaratne, 2007) have been reported to not affect the productivity compared to commercial diets.
Faba beans	Feed intake, egg production, egg weight and food conversion ratio did not change after inclusion of 250 g/ kg faba beans in a laying hens' diet (reducing wheat and soybean meal and increasing sorghum compared to the control diet) (Farrell, Perez-Maldonado, et al., 1999).
Field peas	Contains tannins. Crude protein content of 196 g/ kg fresh field peas of which 13.0 g/ kg lysine (Farrell, Perez-Maldonado, et al., 1999).
Gliricidia sepium seed meal	Crude protein content of 25.55% with lysine at 1.29% DM and high in Iron but tannins, HCN and phenols present (Mutayoba et al., 2011)
Leucaena leucocephala leaf meal	Crude protein content of 29.38% with lysine at 1.47%DM and high in manganese. But NDF and AFD high and tannins, phenols and HCN present (Mutayoba et al., 2011)
Maize bran	Crude protein content at 15.36% but lysine at 0.53% DM only and high in iron (Mutayoba et al., 2011).
Moringa oleifera leaf meal	Crude protein content of 30.65% and lysine 1.40%DM. But phenols and tannins present (Mutayoba et al., 2011).
Musca domestica larvae meal	For broiler chickens between 3 and 6 weeks of age maggot meal can completely replace fishmeal on equi-protein basis in their diets without effect on weight gain, feed consumption and feed conversion (Awoniyi et al., 2003). Inclusion of 20% maggots in the diet of broiler chickens did not affect the feed intake and improved the live weight and the feed conversion. Soybean meal was higher in the control diet but corn, however, was lower (Hwangbo et al., 2009). An inclusion of 12% house fly larvae meal, grown on municipal organic waste, compared to a soybean meal diet did not affect final weight or feed conversion (Ocio et al., 1979).
Palm kernel meal	Contains no anti-nutritional factors but is high in fibre. Immune-boosting and prebiotic effect. Palm kernel meal can included at 40% in broiler diets, but methionine and lysine need to be supplemented (Sundu et al., 2006).

Pearl millet	The performance and carcass yield of broilers fed diets containing up to 50% pearl millet were equivalent or better compared to broilers fed typical corn-soybean diets (Davis et al., 2003). For laying hens, pearl millet can substitute for wheat, sorghum and some soybean meal without affecting egg production, feed conversion or egg weight (Singh et al., 2000).
Red sorghum	Crude protein content of 11.19% with lysine 0.22% DM only. Rich in Ca, Na, S and Cu (Mutayoba et al., 2011).
Shrimp waste meal	Contains 44% of crude protein on DM base. Shrimp waste meal can be included up to 10% in both starter and finisher broiler diets (Okoye et al., 2005).
Sunflower products	Sunflower seed cake meal contains a crude protein content of 31.4% and lysine at 1.02% DM. High contents of NDF and ADF. High in Zn and Cu (Mutayoba et al., 2011). High-fibre sunflower cake increased ileal digestibility of fat and protein and decreased *Clostridium* spp. in the jejunum. Feed conversion was not affected by 20% inclusion of high-fibre sunflower cake (Kalmendal et al., 2011). Replacing soybean meal and maize in layers' diet up to 10% will not affect feed consumption or hen-day egg production. Egg parameter were never (up to 15% high fibre sunflower meal) affected (Rezaei and Hafezian, 2007).
Sweet lupins	Feed intake, egg production, egg weight and food conversion ratio did not change after inclusion of 250 g/ kg sweet lupins in a laying hens' diet (reducing wheat and soybean meal and increasing sorghum compared to the control diet) (Farrell, Perez-Maldonado, et al., 1999).
White sorghum (low tannin)	Crude protein content of 13.65% but lysine at 0.27% DM only (Mutayoba et al., 2011).

Appendix 2: overview of reported bacteria in the chickens' cecum.

	Phylum	Class	Order	Family	Genus	References
Prokaryota-bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Sporomusa	4.87% [2]; 2.2% [17]
		Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma	1,77% [2]
		Clostridia (5% [7])	Clostridiales	Lachnospiraceae	Syntrophococcus	[4]
					Roseburia	[4]
					Coprococcus	[4]
					Anaerofilum	[4]
					Acetitomaculum	[4]
				Clostridiaceae	Ethanoligenens	[4]
					Hespellia	[4]
					Fastidiosipila	[4]
					Coprobacillus	[4]
					Lutispora	[4]
					Clostridium	2.1% [12], 36.4% [8], 62.4% [2], [1], [3], [5], [10], [13], [15]
				Eubacteriaceae	Eubacterium	10.0% [8], 15.5% [2], 60.6% [12]
				Ruminococcaceae	Gemmiger	3,4% [12]
					Oscillibacter	[4]
					Hydrogenoanaerobacterium	[4]
					Ruminococcus	16,4% [8]
				Blautia		[4]
		Bacilli	Lactobacilales	Enterococcaceae	Enterococcus	1.17% [8], 1.33% [2], [4], [5]
				Lactobacilaceae	Lactobacillus	0.79% [8], 5.31% [2], [1], [3], [4], [5], [10], [13], [15]
				Leuconostocaceae	Weissella	0.48% [8]
				Streptococcaceae	Streptococcus	0.65% [8], 0.7% [12], 0.9% [2]
			Bacillales	Bacilaceae	Bacillus	1.42% [8]
		Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae		[6]
	Proteobacteria (3.5% [2])	Gammaproteobacteria	enterobacteriales	enterobacteriaceae	Salmonella	[1]
					Escherichia	1.26% [8], [1], [2], [3], [4], [5], [9], [15]
		Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobacterium	0.79% [8]
		Betaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenes	[8]
					Achromobacter	0.64% [8]
		Epsilonproteobacteria	Campylobacterbacterales	Campylobacteraceae	Campylobacter	[1]
		Deltaproteobacteria	Desulfovibrionales	Desulfuvibrionaceae		[14]
	Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	Bacteroides	5.1% [8], 12.8% [12], [3]
		Cytophagia	Cytophagales	Flammeovirgaceae	Perexilibacter	[4]
					Reichenbachiella	[4]
		Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	0.16% [8]
	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	6.2% [12], 13,5% [8]
	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	[3], [10], [15]
			Coriobacteriales	Coriobacteriaceae	Eggerthella	[16]
Archaea-Euryarchaeota	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter woesei	[11]

Overview of reported bacteria in the chickens' cecum (based on [1] Amit-Romach et al., 2004; [2] Bjerrum et al., 2006; [3] Cao et al., 2005; [4] Danzeisen et al., 2011; [5] Engberg et al., 2004; [6] De Maesschalck et al., 2010; [7] Gong et al., 2007; [8] Lu et al., 2003; [9] Mathlouthi et al., 2002; [10] Mountzouris et al., 2007; [11] Saengkerdsub et al., 2007; [12] Salanitro et al., 1978; [13] Shakouri et al., 2009; [14] Videnska et al., 2014; [15] Xia et al., 2004; [16] Yin et al., 2010; [17] Zhu et al., 2002).

Appendix 3: Articles not included in Chapter 3, study origin and the reason why it was not included

Article	**Country**	**Reason why it was not included**
(Ali, 2002)	Bangladesh	masterthesis; crop and gizzard content mixed
(Almeida et al., 2012)	Denmark	any crop content, different from herbage and supplementary feed, was omitted from the analysis
(de Vries, 2000)	Nicaragua	no qualitative information
(Hanyani, 2012)	South-Africa	masterthesis
(Horsted et al., 2007)	Denmark	controlled forage vegetations
(Kingori et al., 2007)	Kenya	controlled free-range diet
(Kondombo et al., 2003)	Burkina Faso	overall results for different supplemented feeds
(Lomu et al., 2004)	Australia	sample size too small (n = 2)
(Abou-Elezz Fouad Mohammed et al., 2012)	Mexico	concentrated feed available at libitum and restriced scavenge area
(Rahman et al., 2006)	South-East Asia	crop and gizzard content mixed
(Savory et al., 1978)	Scotland	analysis performed on fecal matter
(Wilson, 1965)	Zimbabwe	sample size too small (n = 2)
(Ukil, 1992)	Bangladesh	masterthesis, crop and gizzard content mixed

Appendix 4: Articles used to estimate nutrient composition in commercial broiler and laying hen diets

Commercial broiler diets	**Commercial laying hen diets**
(Awad et al., 2009)	(Chowdhury et al., 2005)
(Çabuk et al., 2004)	(Donalson et al., 2005)
(Cerrate et al., 2011)	(Grobas et al., 2001)
(Kalavathy et al., 2003)	(Keshavarz, 2003)
(Kalmar et al., 2011)	(Lammers et al., 2008)
(Lee et al., 2003)	(Liebert et al., 2005)
(Onderci et al., 2006)	(Roberts et al., 2007)
(Pauwels, Coopman, et al., 2015)	(Schreiner et al., 2004)
(Yang et al., 2008)	(Steenfeldt et al., 2007)
(Zulkifli et al., 2000)	(Zaghini et al., 2005)

Appendix 5: Questionnaire to chicken owners

This questionnaire is part of the PhD research of Jana Pauwels at the Faculty of Veterinary Medicine, Ghent University. With these questions about your chickens I want to get an idea about the costs and the benefits of keeping chickens in a non-commercial way. We are especially interested in alternative food resources for chickens. There are no wrong answers; we only ask that you complete this questionnaire as honest as possible.

Thank you very much for your cooperation!

Jana

Date of interview: ……………………

Identification owner :

- In which year where you born? ………………………………………………………
- Male/female
- What is your profession? ……………………………………………………………..
- What is your highest grade of education? ……………………………………………………………..
- In which village or city are your chickens kept? ……………………………………………………………..

How many adult (older than 6 months) chickens do you currently have ? …………….
How many chicks (younger or at the age of 6 months) do you currently have ? ………

If you have no chickens, please complete question one only.
If you have one or more chickens, please complete all questions.

Thank you very much

1. There are many reasons why people have a limited number of chickens. Can you indicate to which extent the following reasons contribute to the amount of chickens in your flock? I don't have more chickens because:

	1 totally disagree	2 disagree	3 slightly disagree	4 neutral	5 slightly agree	6 agree	7 totally agree
It's too expensive to buy new chickens	☐	☐	☐	☐	☐	☐	☐
It's too expensive to feed more chickens	☐	☐	☐	☐	☐	☐	☐
It's too much work to take care of more chickens	☐	☐	☐	☐	☐	☐	☐
I just started keeping chickens	☐	☐	☐	☐	☐	☐	☐
I don't have enough space	☐	☐	☐	☐	☐	☐	☐
I already have too many chickens	☐	☐	☐	☐	☐	☐	☐
The adult chickens often die	☐	☐	☐	☐	☐	☐	☐
The chicks often die	☐	☐	☐	☐	☐	☐	☐
I have enough chickens	☐	☐	☐	☐	☐	☐	☐

2. People have many reasons to keep chickens. These reasons depend on many things and can vary in time for the same person. Which are your reasons to keep chickens?

	1 totally disagree	2 don't agree	3 moderately disagree	4 neutral	5 moderately agree	6 agree	7 totally agree
Eggs for sale	☐	☐	☐	☐	☐	☐	☐
Eggs for own consumption	☐	☐	☐	☐	☐	☐	☐
Meat for sale	☐	☐	☐	☐	☐	☐	☐
Meat for own consumption	☐	☐	☐	☐	☐	☐	☐
To have a backup in case I need money	☐	☐	☐	☐	☐	☐	☐
To show my wealth	☐	☐	☐	☐	☐	☐	☐
I use the feathers	☐	☐	☐	☐	☐	☐	☐
As a hobby	☐	☐	☐	☐	☐	☐	☐
They eat my kitchen waste	☐	☐	☐	☐	☐	☐	☐
To give them away as a gift	☐	☐	☐	☐	☐	☐	☐

3. A lot of factors can cause death among your chickens. Which causes of death have you noticed among your chickens and chicks in the last two years?

	1 never	2 very rarely	3 rarely	4 sometimes	5 frequently	6 very frequently	7 always
Disease	☐	☐	☐	☐	☐	☐	☐
Starvation	☐	☐	☐	☐	☐	☐	☐
Thirst	☐	☐	☐	☐	☐	☐	☐
Predation	☐	☐	☐	☐	☐	☐	☐
Theft	☐	☐	☐	☐	☐	☐	☐
Slaughter	☐	☐	☐	☐	☐	☐	☐
Old age	☐	☐	☐	☐	☐	☐	☐
They disappear	☐	☐	☐	☐	☐	☐	☐
Cause of death unknown	☐	☐	☐	☐	☐	☐	☐
None of my chickens died in the last two years	☐	☐	☐	☐	☐	☐	☐

4. How many people help you to take care of the chickens?.....................................

5. How much time do you and your helpers spend on the chickens? ……………….

6. If you would have 10 newborn chicks, how many of them do you expect to reach maturity (6 months)?………………………

7. In some villages the chickens run around freely. They can easily mix with the chickens of the neighbors. How do you recognize your chickens?

	1 totally disagree	2 disagree	3 moderately disagree	4 neutral	5 moderately agree	6 agree	7 totally agree
I know them individually	☐	☐	☐	☐	☐	☐	☐
By color	☐	☐	☐	☐	☐	☐	☐
They come to me	☐	☐	☐	☐	☐	☐	☐
They are fenced	☐	☐	☐	☐	☐	☐	☐
There are no other chickens around	☐	☐	☐	☐	☐	☐	☐
I don’t know them	☐	☐	☐	☐	☐	☐	☐

8. People often combine a job with keeping chickens. How do you experience the impact of keeping chickens on your daily workload?

no impact	negligible	reasonable	slightly heavy	exhausting	very exhausting	Full time job
☐	☐	☐	☐	☐	☐	☐

9. Some people keep exact record of their chickens' prestations while other people have totally no idea about it or only follow some prestations e.g. number of eggs, weight, age,…. Which prestations do you record and what is the reason for not recording the other prestations?

	1 totally don't agree	2 don't agree	3 moderately disagree	4 neutral	5 moderately agree	6 agree	7 totally agree
I have no interest	☐	☐	☐	☐	☐	☐	☐
I have no time	☐	☐	☐	☐	☐	☐	☐
It's too difficult	☐	☐	☐	☐	☐	☐	☐
I never thought of it	☐	☐	☐	☐	☐	☐	☐
I don't see the benefits	☐	☐	☐	☐	☐	☐	☐
I weigh my chickens	☐	☐	☐	☐	☐	☐	☐
I observe them daily	☐	☐	☐	☐	☐	☐	☐
I keep record of the number of eggs	☐	☐	☐	☐	☐	☐	☐
I keep record of the number of chicks	☐	☐	☐	☐	☐	☐	☐
I keep record of the number of chickens	☐	☐	☐	☐	☐	☐	☐
I keep record of my chickens' age	☐	☐	☐	☐	☐	☐	☐

10. Chicken meat is low in fat and rich in protein. If you had to choose a chicken for slaughter, which one would you choose?

	1 never	2 very unlikely	3 unlikely	4 neutral	5 likely	6 very likely	7 always
I would refuse to choose a chicken for slaughter	☐	☐	☐	☐	☐	☐	☐
The oldest hen	☐	☐	☐	☐	☐	☐	☐
The heaviest hen	☐	☐	☐	☐	☐	☐	☐
The oldest rooster, if I have more than one	☐	☐	☐	☐	☐	☐	☐
The heaviest rooster, if I have more than one	☐	☐	☐	☐	☐	☐	☐
The chicken that is easiest to catch	☐	☐	☐	☐	☐	☐	☐
It doesn't matter	☐	☐	☐	☐	☐	☐	☐
The most aggressive one	☐	☐	☐	☐	☐	☐	☐
A young hen that is not laying eggs yet	☐	☐	☐	☐	☐	☐	☐
I would slaughter my own chickens	☐	☐	☐	☐	☐	☐	☐

11. Some chickens have a whole village to live in and other chickens are kept on a small fenced area. To which facilities do your chickens have access?

	1 never	2 very rarely	3 rarely	4 sometimes	5 frequently	6 most of the time	7 always
Natural water supply	☐	☐	☐	☐	☐	☐	☐
Water provided by people	☐	☐	☐	☐	☐	☐	☐
Accessible trees	☐	☐	☐	☐	☐	☐	☐
Bush to hide	☐	☐	☐	☐	☐	☐	☐
Open stable	☐	☐	☐	☐	☐	☐	☐
Predator safe shelter during the night	☐	☐	☐	☐	☐	☐	☐
Unfenced area to scavenge	☐	☐	☐	☐	☐	☐	☐
Fenced area to scavenge	☐	☐	☐	☐	☐	☐	☐
House of humans	☐	☐	☐	☐	☐	☐	☐
Garbage belt or compost belt	☐	☐	☐	☐	☐	☐	☐

12. Some chickens run around freely and are supposed to find their own feed. These chickens can be fed in addition to what they find outside. What do you feed your chickens in addition to what they scavenge?

	1 never	2 very rarely	3 rarely	4 sometimes	5 frequently	6 very frequently	7 always
Kitchen leftovers	☐	☐	☐	☐	☐	☐	☐
Commercial chicken grain mix	☐	☐	☐	☐	☐	☐	☐
Grain from own cultivation	☐	☐	☐	☐	☐	☐	☐
Crops from own cultivation	☐	☐	☐	☐	☐	☐	☐
Collected insects	☐	☐	☐	☐	☐	☐	☐
It depends on the season **what** I feed my chickens	☐	☐	☐	☐	☐	☐	☐
It depends on the season **how much** I feed my chickens	☐	☐	☐	☐	☐	☐	☐
I provide clean water	☐	☐	☐	☐	☐	☐	☐
My chickens could survive on the feed I provide them with, without scavenging	☐	☐	☐	☐	☐	☐	☐
My chickens could survive on what they find during scavenging only	☐	☐	☐	☐	☐	☐	☐

13. Nobody exactly knows what chickens eat if they scavenge around. Which of the following feeds have you noticed are eaten by your chickens?

	1 never	2 very rarely	3 rarely	4 sometimes	5 frequently	6 very frequently	7 constantly
Grains/seeds	☐	☐	☐	☐	☐	☐	☐
Grass	☐	☐	☐	☐	☐	☐	☐
Flies	☐	☐	☐	☐	☐	☐	☐
Mosquito's	☐	☐	☐	☐	☐	☐	☐
Beetles	☐	☐	☐	☐	☐	☐	☐
Worms	☐	☐	☐	☐	☐	☐	☐
Spiders	☐	☐	☐	☐	☐	☐	☐
Ticks	☐	☐	☐	☐	☐	☐	☐
Mice	☐	☐	☐	☐	☐	☐	☐
Leftovers from people	☐	☐	☐	☐	☐	☐	☐
Dead animals	☐	☐	☐	☐	☐	☐	☐
Small stones/ sand/gravel	☐	☐	☐	☐	☐	☐	☐
Others:							

14. Insects are very common were animals live. Some people may even consider them a plaque. Are there bugs that cause a plague where you live?
…………………………………………………………………………………………….

15. If you go to the market or the shop, what do you expect to be the price of 1kg of the cheapest commercial chicken feed? ………………

16. If you go to the market or the shop, what do you think is the normal price for an egg? ……………………………………………

17. This is a 2 year old hen and a 2 year old rooster. How much money will you receive if you sell them on the market?

For the hen:……………… For the rooster: ……………….

18. People make all kinds of investments for their chickens. Some spend a lot of money while others spend none. Where do/did you spend money on for your chickens?

	1 nothing	2 very few	3 few	4 enough	5 a lot	6 very much	7 too much
Feed	☐	☐	☐	☐	☐	☐	☐
Veterinarian	☐	☐	☐	☐	☐	☐	☐
Housing	☐	☐	☐	☐	☐	☐	☐
Medicine	☐	☐	☐	☐	☐	☐	☐
New chickens	☐	☐	☐	☐	☐	☐	☐
Feed and drinking equipment	☐	☐	☐	☐	☐	☐	☐
Protection for predators	☐	☐	☐	☐	☐	☐	☐
Litter	☐	☐	☐	☐	☐	☐	☐
Toys	☐	☐	☐	☐	☐	☐	☐

19. Imagine somebody gave you extra money to spend on your chickens, which things would you immediately change and which ones would you not change?

	1 I would never change it	2 totally unimportant	3 slightly unimportant	4 neutral	5 important	6 very important	7 priority
More food	☐	☐	☐	☐	☐	☐	☐
Vaccination	☐	☐	☐	☐	☐	☐	☐
Better housing	☐	☐	☐	☐	☐	☐	☐
Deworming	☐	☐	☐	☐	☐	☐	☐
New chickens	☐	☐	☐	☐	☐	☐	☐
Protection for predators	☐	☐	☐	☐	☐	☐	☐
I would buy more chickens	☐	☐	☐	☐	☐	☐	☐
I would not change anything	☐	☐	☐	☐	☐	☐	☐

Thank you very much for your cooperation!

Jana

Summary

Summary

Although the percentage of hungry people in the world is decreasing, still one out of ten (nearly 800 million) is undernourished. Nevertheless, commercial chickens are fed a diet that contains over 80% of soybean meal and cereal grains such as wheat and corn. Of the global cereal grain production, 8% goes to poultry feed while cereals can be used in the human diet too. Of the global soybean meal production, 48% is used in poultry diets. Though soybean meal is not a part of the human diet and is, strictly spoken, considered a by-product, soybeans are grown explicitly to produce soybean meal for feed and 1 kg of soybeans is needed to gain 0.8 kg of soybean meal. In addition, industrial chickens induce a loss of human-edible energy and protein while converting feed to eggs and meat. This is in contrast to rural chickens were the efficiency is reported to exceed 100% as these chickens are often fed human-inedible ingredients. Considering the growing population, both of human and livestock, questions about the sustainability of the current intensive feeding system, where food is used as feed, arise. Even if a small decrease of the share of food in the diets of chickens could be achieved, the impact will be significant as there are 21.3 billion chickens in the world, which equals three per person.

When aiming to decrease the use of food in the feed of chickens, the first step is to list alternative ingredients. Knowing alternative, local ingredients that can be used in chicken feed will also benefit rural chicken owners, especially in developing countries, for their dependence of commercial grains will decrease. Observing the scavenging diet of free-ranging chickens (**Chapter 3**) teaches that chickens ingest a huge variety of ingredients such as seeds, green forages, insects and kitchen waste. Seeds are the most common environmental ingredient, irrespective of ingredients supplemented by humans. As the amount of seeds in the crop is positively correlated with the crude fibre content, we learn that chickens do not avoid fibrous ingredients, while fibre is generally considered of low energetic value and, in high amounts, even anti-nutritious. Compared to a commercial diet, the scavenging diet was higher in crude fibre, lower in crude protein, Ca, P and energy and tended to be lower in crude fat. As this was also seen when comparing the nutrient content of the supplemented ingredients to the environmental ingredients, the question raises whether the actual nutrient and energy profile of feeds for

commercial breeds is a consequence of the increased requirements for fast growth or high egg production, or did these breeds develop based on the diet change that was induced by humans?

When aiming to increase the profit of rural chicken owners, knowledge about their chicken management is needed. In general, these chickens are kept in traditional free-range systems and their management (or the absence thereof) differs largely from the management that is considered optimal, based on industrial parameters. As exact information on this traditional management is scarce, a survey among the rural chicken owners in Limpopo, South-Africa, was performed (**Chapter 4**). Limpopo is the poorest province in the country and owning chickens is suggested to be a possible additional source of income and animal protein. We aimed to determine ways to increase the profit for rural chicken owners, without tackling the traditional system. It was concluded that a minimum of time, money and effort was spent on the chickens. The chickens, however, needed both scavenger and supplemented feed to survive. Besides slaughter, the main causes of death were disease and predation, improving the health and the protection of the chickens should be the focus when trying to increase the profit of traditional free-ranging chicken husbandry.

If the fibre content increases by using alternative ingredients, fibre breakdown will be important for the chickens. Fermentation of fibre in chickens is performed by the microbiota in the ceca and literature suggests that the microbiota of indigenous free-range chickens is more capable to break this fibre down compared to industrial chickens. Which raises questions about possibilities to increase fibre fermentation by transplanting cecal microflora. To study these bacteria, so far, chickens always had to be euthanized in order to obtain a cecal sample. In **Chapter 5a**, quantification and identification of the microbial populations in cecal drop, cecal content and fecal drop samples from chickens showed that cecal drop contains a bacterial community that is very similar (concerning bacterial diversity, richness and species composition) to cecal content, as opposed to the bacterial community found in fecal drop. Therefore, a sample from the cecal drop can be used as a reliable representative for the cecal microbiota. Indicator genera for the scavenger and the commercial diet could be identified but interpreting the effects of these genera on the overall health and digestibility of nutrients was not possible (**Chapter 5b**).

As insects are found in each scavenger diet (**Chapter 3**) and as they are known to convert waste streams into protein, fat and micronutrients, they are very interesting alternative ingredients. Before including insects in feed, however, studies on digestibility and nutrient composition will be needed. Chitin is a nitrogen containing polysaccharide that is included in the insects' exoskeleton. On the one hand does the nitrogen in chitin bias the results for crude protein, obtained by the Kjeldahl analysis. On the other hand, crude fibre causes a bias in the chitin analysis. A method, based on the determination of nitrogen in the chitin fraction, was discovered to clarify these entangled results and to estimate true protein more accurately (**Chapter 6**).

Adding alternative ingredients (fibrous, insects or others) that do not compete with the human diet might require changes throughout the whole chicken production system. The growth rate of chicken breeds, for example, is linearly related to their resilience to cope with fibre-rich and protein and energy-poor diets. Meaning that fast growing breeds decrease their weight gain on a scavenger diet, whereas slow growing breeds can keep their growth rate the same for both diets by increasing their feed intake. No differences of digestive capacity between the breeds were observed (**Chapter 7**). Re-selecting chicken breeds for high performance on sustainable diets might increase the final profit.

To increase the use of human-inedible ingredients in feed, more than only the feed might have to change. Dealing with fibre-rich ingredients, exploring ways to add sustainably bred insects to feed and selecting chicken breeds to produce on less concentrated diets are, by this PhD work, found to be the topics that need priority. Today, chickens on less energy dense diets will not exceed the profits made in the intensive poultry industry on concentrated diets. Yet, as the feed prices increase over the years, a tipping point might arrive where a lower production is justified by the lower costs of feed ingredients. Anyway, considering the global population growth, increasing the production of animal products will be necessary. The question remains how to achieve this within the finite sources of our planet.

Samenvatting

Samenvatting

Alhoewel het percentage ondervoede mensen in de wereld daalt, is vandaag de dag nog steeds één op tien mensen (800 miljoen in totaal) ondervoed. Toch bestaat het voeder van kippen in de industrie voor 80% uit sojaschroot en granen zoals tarwe en maïs. Acht procent van de globale graanproductie gaat naar kippenvoeder terwijl granen ook direct door mensen geconsumeerd kunnen worden. Van de algemene productie van sojaschroot gaat zelfs 48% naar kippenvoeder. Alhoewel sojaschroot niet direct door mensen geconsumeerd wordt en dus, strikt genomen, een bijproduct is, worden sojabonen geproduceerd met het specifieke doel om er sojaschroot voor diervoeders van te maken. Om 0.8 kg sojaschroot te maken, heeft men 1 kg sojabonen nodig. Bovendien gaan er voor de mens beschikbare eiwitten en calorieën verloren wanneer commerciële kippen hun voeder omzetten in vlees en eieren, in tegenstelling tot kippen die gevoederd worden met ingrediënten die voor de mens niet benutbaar zijn. Aangezien de populatie van zowel mens als vee groeit, wordt de duurzaamheid van dit intensieve voeder systeem, waarbij voedsel als voeder wordt gebruikt, in vraag gesteld. Zelfs een kleine besparing op het aandeel "voedsel" in het voeder van kippen, zou al een groot verschil maken daar er 21.3 miljard kippen zijn in de wereld, ofte een drietal per persoon.

In het streven naar een lager gebruik van voedsel als voeder bij kippen, is het een eerste stap om mogelijke alternatieven op te sommen. Kennis over het gebruik van lokale bronnen in kippenvoeder is bovendien belangrijk voor landelijke boeren, vooral in ontwikkelingslanden, daar zij hierdoor minder afhankelijk zullen worden van granen op de commerciële markt. Het observeren van loslopende, scharrelende kippen (**Hoofdstuk 3**) wijst uit dat kippen een grote variëteit aan ingrediënten consumeren zoals zaden, kruiden, grassen, insecten en keukenafval. Zaden werden het meest geconsumeerd, los van de ingrediënten die door de mens gesupplementeerd werden. Aangezien de hoeveelheid zaden in de krop positief gecorreleerd was met het aandeel ruwe celstof, leerden we dat kippen geen vezelachtige substraten vermijden, terwijl vezels in kippenvoeder als laag energetisch beschouwd worden en, in grotere hoeveelheden, zelfs de voederwaarde van het dieet doen dalen. In vergelijking met een commercieel dieet bevatte het scharreldieet meer ruwe celstof, minder ruw eiwit, Ca, P en energie, en neigde lager te zijn in ruw vet. Aangezien dit ook opgemerkt werd wanneer nutriëntensamenstelling van natuurlijke ingrediënten vergeleken werd met door de mens gesupplementeerde ingrediënten, kan men zich afvragen of de hedendaagse voeder- en energiewaarden in commerciële voeders het gevolg zijn van behoeften voor snelle groei en hoge eiproductie of juist aanleiding heeft gegeven tot de ontwikkeling van deze hoogproductieve rassen.

Wanneer getracht wordt de opbrengst van landelijke kippenhouders in ontwikkelingslanden op te krikken, is informatie over hun management onontbeerlijk. Traditioneel worden deze kippen volledig vrij gehouden en hun management (of de afwezigheid ervan) verschilt in grote mate van het optimale, bepaald op basis van industriële parameters. Daar exacte informatie over dit management zeldzaam is, werd een enquête afgenomen bij de lokale kippenhouders in Limpopo, Zuid-Afrika (**Hoofdstuk 4**). Limpopo is de armste provincie in Zuid-Afrika en het houden van kippen kan de bevolking voorzien van extra geld en dierlijke eiwitten. Ons doel was om mogelijkheden te identificeren waarop kippenhouders hun inkomsten via de kippen konden verhogen zonder in conflict te treden met het traditionele beleid. Er werd geconcludeerd dat een minimum aan tijd, geld en moeite aan de kippen werd gespendeerd. Toch hebben deze kippen zowel het voeder dat ze vinden in de natuur als het voeder dat ze van de mensen krijgen nodig. Als slachten niet wordt meegerekend, stierven kippen voornamelijk aan een ziekte of door predatie. Dit suggereert dat, om de inkomsten van de traditionele kippenhouderij in ontwikkelingslanden te verhogen, de klemtoon moet liggen op het verbeteren van de gezondheid van de kippen en het beter beschermen tegen roofdieren.

Als de vezelinhoud van voeders verandert door het gebruik van alternatieve ingrediënten, zal vezelafbraak voor kippen belangrijker worden. Bij kippen worden diëtaire vezels gefermenteerd door de microbiota in de ceca en uit de literatuur blijkt dat de microbiota van rurale, scharrelende kippen beter in staat is om vezels af te breken dan van industriële kippen. Dit roept uiteraard vragen op over de mogelijkheden van het transplanteren van cecale microbiota. Om het cecale microbioom van kippen te bestuderen werden, tot de dag van vandaag, kippen geëuthanaseerd om een staal uit het cecum te bekomen. In **Hoofdstuk 5a** werd de microbiële populatie in cecale drops, cecale inhoud en fecale drops geïdentificeerd en gekwantificeerd. Hieruit bleek dat de bacteriële samenstelling in cecale drops zeer gelijkaardig is aan de bacteriële samenstelling in de ceca (aangaande diversiteit, rijkdom en species samenstelling), in tegenstelling tot de bacteriële samenstelling in fecale drops. Dit zorgt ervoor dat een staal van de cecale drop als een betrouwbare vertegenwoordiger kan beschouwd worden van de cecale microbiota. Enkele genera werden aangetoond als specifieke indicatoren voor het scharreldieet maar het is onmogelijk om hun effect op de gezondheid en verteerbaarheid te bepalen op basis van onze data (**Hoofdstuk 5b**).

In elk scharreldieet werden insecten gevonden en aangezien insecten afvalstromen kunnen omzetten naar eiwitten, vet en micronutriënten worden zij als zeer interessante alternatieve ingrediënten beschouwd. Vooraleer insecten in voeders gebruikt kunnen worden, zullen er echter studies nodig zijn naar hun voedingswaarde en verteerbaarheid. Chitine is een stikstof bevattend polysaccharide in het exoskelet van insecten. Aan de ene kant interfereert stikstof in chitine met de bepaling van ruw eiwit aangezien ruw eiwit geanalyseerd wordt op basis van totale stikstof met de Kjeldahl methode. Aan de andere kant vertekent ruwe celstof in het staal het chitine resultaat. In **Hoofdstuk 7** werd een methode bepaald, op basis van de bepaling van stikstof in de chitine fractie, om deze resultaten te ontwarren en daardoor ruw eiwit accurater te kunnen bepalen.

Alternatieve ingrediënten incorporeren, die niet in competitie treden met de voeding van de mens, zal veranderingen vergen doorheen het hele productieproces van de kip. Zo is de groeisnelheid van kippenrassen bijvoorbeeld, lineair gecorreleerd met hun flexibiliteit om te presteren op vezelrijke en eiwit- en energiearme voeders. Dit betekent dat snel groeiende rassen minder in gewicht toenemen wanneer ze een scharreldieet gevoederd worden. Dit in tegenstelling tot traag groeiende rassen die erin slagen hun gewicht op peil te houden door hun voederopname te verhogen. Er werden geen verschillen in verteringscapaciteit gevonden tussen de verschillende rassen (**Hoofdstuk 7**). Een selectie van kippenrassen die sterk presteren op duurzame voeders kan de finale inkomsten verhogen.

Om meer ingrediënten, niet consumeerbaar voor de mens, te gebruiken in voeders, zal er meer moeten veranderen dan enkel het voeder. Dit doctoraatswerk benadrukt het belang van strategieën om hogere vezelgehaltes in het kippenvoeder te incorporeren, van onderzoek naar manieren om duurzaam gekweekte insecten toe te voegen in kippenvoeder en van kippenrassen te selecteren die sterk produceren op minder geconcentreerde voeders. Vandaag de dag zullen kippen op minder geconcentreerde diëten niet méér inkomsten genereren dan in de industrie waar geconcentreerde voeders gebruikt worden. Maar, aangezien de voederprijzen stijgen doorheen de jaren, wordt er een kantelpunt verwacht waarop een lagere productie gerechtvaardigd zal worden door de lagere kosten van de alternatieve ingrediënten. In ieder geval zal de vraag naar dierlijke eiwitten blijven stijgen daar de wereldbevolking blijft toenemen. De vraagt blijft hoe dit te realiseren binnen de beperkte mogelijkheden van onze planeet.

Curriculum vitae

Curriculum vitae

Jana Pauwels werd geboren op 21 augustus 1986 in Eeklo. In 2004 behaalde zij haar diploma secundair onderwijs in de richting Latijn-Wiskunde(8) aan Nieuwen Bosch te Gent. Nadien startte zij haar universitaire studies en behaalde in 2011 haar diploma diergeneeskunde aan de universiteit van Gent met onderscheiding.

In 2013 kreeg zij een VLIR-UOS beurs toegewezen en startte zij als doctoraatsstudent aan de vakgroep Voeding, Genetica en Ethologie. Gedurende 3.5 jaar onderzocht zij het gebruik van duurzame ingredienten in kippenvoeder. Haar onderzoek verliep in samenwerking met de Universiteit van Limpopo in Zuid-Afrika. Zij begeleidde meerdere studenten, zowel in België als in Zuid-Afrika.

Jana Pauwels is auteur en medeauteur van meerdere publicaties in internationale wetenschappelijke tijdschriften en was meermaals spreker op internationale congressen.

Curriculum vitae

Jana Pauwels was born on August 21, 1986 in Eeklo. In 2004, she obtained her diploma of secondary education in Latin-Mathematics(8) at Nieuwen Bosch in Ghent. In 2005, she started her studies in Veterinary Medicine at Ghent University and graduated in 2011 with distinction.

In 2013 she obtained a VLIR-UOS scholarship and started as PhD student at the department of Nutrition, Genetics and Ethology. For 3.5 years, she investigated the use of sustainable ingredients in chicken feed in cooperation with the University of Limpopo, South-Africa. She supervised several students, both in Belgium and in South-Africa.

Jana Pauwels has authored and co-authored several publications in international scientific journals and was repeatedly speaker at international conferences.

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Dankwoord

Dankwoord

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It always seems impossible,
until it’s done.

Nelson Mandela